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Effects of Prolonged Sitting on Normal, Exercise-Induced Metabolic Improvements

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Effects of Prolonged Sitting on Normal, Exercise-Induced Metabolic Improvements

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Abstract

Effects of Prolonged Sitting on Normal, Exercise-Induced Metabolic Improvements

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Cardiovascular disease (CVD) is an ever-growing cause of mortality and has been coupled with a rise in sedentary behavior. A vast majority of people spend their time physically inactive with the occasional bout of acute exercise. Generally, acute exercise is able to improve postprandial lipemia (PPL), a risk factor for CVD. However, research is beginning to suggest that sedentary behavior might abolish the metabolic benefits normally seen from exercise. This study set out to elucidate the impact of an acute bout of exercise on PPL after four days of prolonged sitting (~13.5 h/day). Subjects participated in a counterbalanced, crossover study in which they completed two trials: prolonged sitting without exercise (SIT) and prolonged sitting with a one-hour bout of treadmill exercise (SIT+EX). Following each trial, plasma triglycerides and glucose were obtained and substrate oxidation via indirect calorimetry was collected to be analyzed for possible improvements caused by exercise. No differences ($p>0.05$) were found in triglyceride or glucose response during the high fat tolerance test, evidenced by triglyceride or glucose AUC_t or AUC_i, or fat oxidation as measured by indirect

calorimetry between trials. While the triglyceride temporal response was similar to expectation with a rise to peak around hour 3-4 postprandial, a noticeably flatter and more prolonged response was seen in the glucose temporal response. This lack of difference between trials comes with similar activity except for the one-hour bout of exercise. The results from this study suggest that prolonged sitting imposes some sort of resistance to the normal improvement in PPL and fat oxidation after acute exercise. This suggests that physical inactivity (e.g. prolonged sitting) creates a condition whereby people are resistant to the normal metabolic improvements in fat metabolism that are derived from a bout of acute exercise.

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Chapter 1: General Introduction

1.1 Background

The modernization of society has led to vast technological advancements that have improved the quality of life and the longevity of the population across the world. However, with a modernizing society come serious problems in that more people are spending their time sedentary. Couple this with improvements in agriculture and sustainability and people are spending more time sitting in a fed state. Along with this rise in sedentary behavior has come an increase in the mortality rate as caused by cardiovascular disease (National Center for Health Statistics, 2016). This phenomenon may be driven by the rise in atherosclerosis driven by postprandial sedentary behavior. As early as 1979, atherosclerosis was described as a "postprandial phenomenon" (Zilversmit, 1979). Rising plasma triglyceride concentration after a meal (Dash, Xiao, Morgantini, & Lewis, 2015; Goldberg, 2009; Hussain, 2000) may be driving the pathogenesis of atherosclerosis.

Exercise has been a well-documented mechanism to attenuate the rise in PPL (Beidleman et al., 2008; Herd, Kiens, Boobis, & Hardman, 2001; Malkova et al., 2000; Zhang, Thomas, & Ball, 1998) However, despite well-established (Garber et al., 2011) and newly formed recommendations (Ekelund, Steene-Johannessen, & Brown, 2016) for exercise, the exercise performed may not be enough to overcome the detrimental effects of sedentary time. New evidence is beginning to suggest that prolonged sitting and sedentary time may reverse or impair the positive effects generally caused by exercise (Duvivier et al., 2013; Kim, Park, Chou, Trombold, & Coyle, 2016). In order to counteract this so-called exercise resistance, higher levels of physical activity throughout the day may be needed. In fact, many studies are now pointing to the idea that breaking

up sedentary time, independent of total moderate to vigorous physical activity, may be able to attenuate PPL as compared to their sedentary counterparts (Duvivier et al., 2013; Healy et al., 2008; Peddie et al., 2013). It is thus imperative to determine if prolonged sitting does limit the effectiveness of an acute bout of exercise to reverse impaired PPL, as a resistance may demonstrate that acute exercise when engaging in otherwise sedentary behavior has little effect on improving PPL (Kim et al., 2016). The goal of this study is to quantify how PPL responds to prolonged sitting with and without an acute bout of moderate intensity exercise.

1.2 Research Purpose and Hypothesis

The goal of this study is to determine the impact of a single bout of acute exercise on plasma triglycerides and glucose, as well as fat oxidation after an acute bout of prolonged sitting. Two groups will undergo the prolonged sitting, but only one will perform the exercise. The specific hypothesis is as follows:

1. A single bout of 1-hour of running at 65% VO_{2max} after prolonged sitting (>14 h/d) will not produce significantly different postprandial triglyceride responses as compared to the control of just sitting.

Chapter 2: Review of Literature

2.2 Introduction

Ensuring individuals live long, healthy lives is a central tenet of modern medicine. However, despite the astounding technological advances that the world has witnessed over the past century, medical treatment is not a fully effective solution for many disorders and needs to be coupled with active prevention. Over the past few decades, heart disease has constantly been the number one cause of mortality in the United States constituting nearly one-quarter of all deaths (National Center for Health Statistics, 2016). Between 1980 and 2014, heart disease saw a reduced contribution to mortality and saw all-cause cancer narrow the margin leaving the two as equals in terms of contribution to mortality (National Center for Health Statistics, 2016). However, it appears that heart disease is still a serious problem as it outnumbers all other causes of death by more than four-to-one (National Center for Health Statistics, 2016).

As heart disease acts as a subcategory of general cardiovascular disease, the risk factors that contribute to heart disease should also contribute to cardiovascular disease. Of all the factors that contribute to cardiovascular disease, atherosclerosis is perhaps the most important (Libby, 2002). Atherosclerosis has numerous sources that contribute to its development, one of which is the presence of abnormal blood lipids (Mozaffarian et al., 2015; Nordestgaard, Benn, Schnohr, & Tybjaerg-Hansen, 2007; Zilversmit, 1979). However, it seems as though some parties may be quick to suggest pharmaceutical intervention (Bansal et al., 2007) when it seems that inactivity may be an easily correctable factor that can reduce the risk of atherosclerosis (Laufs et al., 2005; Mozaffarian et al., 2015). Modern society presents all the factors for a perfect environment leading to atherosclerosis including modern technology allowing for a sedentary lifestyle and an abundance of food that influence the body's lipids and

endothelial function. Without proper intervention, such as adequate exercise or physical activity, cardiovascular disease will continue to be a serious problem in modern medicine.

2.2 HYPERTRIGLYCERIDEMIA AND ATHEROSCLEROSIS

Blood lipids can be measured during either the fasting or postprandial states, both of which offer insight into the triglyceride connection to cardiovascular disease. Several groups have found relationships between fasting triglyceride levels and cardiovascular disease (Assmann, Schulte, & von Eckardstein, 1996; Jeppesen, Hein, Suadicani, & Gyntelberg, 1998; Manninen et al., 1992). High fasting triglyceride levels may be indicative of the body's inability to clear triglycerides and leave them in circulation, which may cause downstream problems to be discussed later. However, it has been suggested that postprandial triglyceride levels are more strongly related to cardiovascular events than fasting triglyceride levels even when considering other factors (Bansal et al., 2007; Nordestgaard et al., 2007). How elevated triglycerides occur and how they influence the formation of atherosclerosis is a multi-faceted explanation that includes both the sources and the physiological mechanisms attenuating clearance.

2.2.1 Sources of Triglyceride

Triglyceride can be derived from two different sources: exogenous and endogenous. Exogenous triglyceride is derived from dietary sources while endogenous triglyceride is synthesized within the liver (Goldberg, 2009). Both sources of triglyceride are composed of generally the same subunits composed of triglyceride, phospholipid, cholesterol, and cholesteryl ester in varying concentrations (Nichols, 1969). The

difference between exogenous and endogenous sources of triglyceride lies in how the lipid is delivered. In the case of exogenous sources, triglyceride is delivered predominantly via chylomicrons, while endogenous sources are delivered via very low-density lipoproteins (VLDLs) (Goldberg, 2009; Nichols, 1969).

As mentioned, chylomicrons are derived from dietary triglyceride intake and begin their life in the intestine as apolipoprotein B48 which is synthesized into and secreted as chylomicron. This assembly is not a constant process, however, and it has been suggested that it is only induced in the postprandial state. In addition, it appears that chylomicron production changes depending on the type of lipid consumed with saturated fat producing the greatest response and polyunsaturated fat producing the least response. These chylomicrons then serve to attach lipids that have been reassembled from contents hydrolyzed and absorbed in and from the lumen (Dash et al., 2015; Hussain, 2000). Chylomicrons are then secreted into the lymphatic system by the small intestine to begin delivery of lipids to the central circulation (Goldberg, 2009). This highly regulated process is important to ensure adequate triglyceride clearance. Approximately 95% of the 100-150 g/day of triglycerides that adults consume is handled by chylomicrons of various sizes and compositions (Dash et al., 2015; Hussain, 2000). Given that chylomicrons are generally only synthesized and secreted in the postprandial state, fasting levels of chylomicron triglycerides should be lower. However, in certain diseases that present hypertriglyceridemia or hyperlipidemia such as diabetes, hyperchylomicronemia can occur in the fasted state due to increased VLDL levels and saturation of lipoprotein lipase (LPL), which will be discussed later (Dash et al., 2015; Goldberg, 2009; Zilversmit, 1979).

Derived in the liver, VLDLs are the major delivery method of triglycerides in the fasted state. While chylomicrons are dependent on apolipoprotein B48, VLDLs are

dependent on apolipoprotein B100 (Ginsberg, Zhang, & Hernandez-Ono, 2005; Hussain, 2000). The formation of VLDL triglycerides is a substrate driven process reliant upon the concentration of hepatic free fatty acids (FFAs) (Nielsen & Karpe, 2012). Triglycerides attached to VLDL may also be of a carbohydrate origin that have been converted to triglyceride via *de novo* lipogenesis. This source of triglyceride may not be appreciable with dietary changes, however, unless the addition of dietary carbohydrate greatly exceeds total energy expenditure (Hellerstein, 1999; Nichols, 1969). Unlike chylomicrons, VLDL is constantly being produced to provide sources of triglycerides to different bodily tissues (Hussain, 2000). VLDL production is also influenced by certain disease states. Diabetes, particularly insulin resistant diabetes, leads to an increased secretion of VLDL into circulation. This increase in VLDL adds to the hypertriglyceridemia and hyperlipidemia presented via the increase in chylomicron circulation (Ginsberg et al., 2005; Lewis & Steiner, 1996; Nielsen & Karpe, 2012).

Uptake and metabolism of chylomicrons and VLDL are regulated by a similar mechanism in LPL, as mentioned previously. LPL is an enzyme located on the luminal side of vascular endothelial cells in adipose, skeletal muscle, and myocardial tissue (Dash et al., 2015; Wang & Eckel, 2009). Metabolism of both chylomicrons and VLDLs are closely regulated by the ratios of apolipoproteins CII and CIII that have been acquired after these carriers have been circulated in the lymph and blood (Dash et al., 2015; Ginsberg et al., 2005). LPL subsequently hydrolyzes the triglyceride attached to chylomicron and VLDL sources to allow for uptake of fatty acid into adjacent cells. Aside from fatty acids, other remnants are produced by the hydrolysis process, namely low-density lipoproteins (LDLs) as an end product (Ginsberg et al., 2005). VLDLs may also undergo structural changes in the blood as lipid transfer proteins and hepatic lipase increase the production of LDL (Goldberg, 2009). This production of LDL and other

remnants are thought to be the major factors leading to the pathogenesis of atherosclerosis (Zilversmit, 1979).

2.2.2 Mechanisms of Hypertriglyceridemia and their Link to Atherosclerosis

As previously established, a strong association between hypertriglyceridemia and cardiovascular disease has been recognized (Assmann et al., 1996; Jeppesen et al., 1998; Manninen et al., 1992). Despite debate over the cause of atherosclerosis, the most common explanation for the onset of atherosclerotic formation begins with an increased accumulation of lipoprotein behind the endothelial wall of the vasculature (Lusis, 2000; Navab et al., 1995; Williams & Tabas, 2005). These molecules are then rapidly trapped in the subendothelial space through interactions with apolipoprotein B and matrix proteoglycans (Lusis, 2000; Navab et al., 1995; Tabas, Williams, & Boren, 2007). Once trapped, LDL undergoes modification via oxidation, lipolysis, proteolysis, and aggregation. All of these changes lead to the formation of foam cells via macrophage infiltration/conversion and to inflammation of the surrounding tissue (Lusis, 2000; Tabas et al., 2007). This damaged tissue and subsequent lipid/cholesterol infiltration eventually heals and develops a fibrous cap consisting of smooth muscle cells. The underlying tissue then forms a necrotic core and the exterior may begin to calcify. However, this area is suspect to further injury, especially if the fibrous cap that forms is particularly thin. In this case, further growth of the lesion or breaking off of the cap and potential release of the interior contents of the lesion can lead to thrombosis (Lusis, 2000; Tabas, 2004). As soon as thrombosis occurs, the risk of cardiovascular events, such as stroke or myocardial infarction, greatly increases.

Though the primary factor for the development of atherosclerotic lesions seems to be LDL, there are other substances that may add to the formation of atherosclerosis. The two molecules, chylomicrons and VLDL, which contribute to LDL formation via LPL hydrolysis and blood conformational changes are not necessarily atherosclerotic on their own. Rather, the remnant products of hydrolysis and lipolysis of chylomicrons and VLDL contribute directly to the accumulation of lipoproteins in the subendothelial space (Tabas, 2004). As the concentration of chylomicrons increases postprandially while VLDL continues to be released, as its production is controlled by liver concentrations of FFAs, the amount of circulating triglyceride and cholesterol is elevated to significant levels (Zilversmit, 1979). As chylomicrons and VLDLs both utilize LPL (Dash et al., 2015; Wang & Eckel, 2009) as an uptake mechanism, postprandial saturation of LPL can occur as the two molecules compete for binding sites. Therefore, this increase in circulating cholesterol/triglycerides and concomitant saturation of LPL allows for the opportunity for chylomicron and VLDL byproducts to build up in the subendothelial space. In addition to this formation of lesions, increased concentrations of VLDL cause corresponding decreases in high density lipoprotein (HDL) concentrations (Ginsberg et al., 2005). This is problematic as HDL is generally believed to have protective effects against atherosclerosis (Ginsberg et al., 2005; Lusis, 2000; Navab et al., 1995; Williams & Tabas, 2005).

2.3 EXERCISE AND HYPERTRIGLYCERIDEMIA

Exercise is commonly recognized to decrease the risk of a variety of systemic diseases and metabolic disorders. Among those that exhibit a reduced prevalence are cardiovascular disease, insulin resistant diabetes mellitus, and select cancers (Beidleman

et al., 2008). Given the possible therapeutic nature that exercise provides for a variety of diseases, it is necessary to explore the cause and effect nature of exercise. In particular, exercise can have an impact on postprandial hyperlipidemia by affecting circulating triglyceride levels (Beidleman et al., 2008; Herd et al., 2001; Malkova et al., 2000; Zhang et al., 1998). By lowering the postprandial triglyceride levels, it may be possible to reduce the risk of atherosclerosis through previously discussed mechanisms, thus making exercise a viable alternative to pharmaceutical treatment.

2.3.1 Factors of Exercise Attenuating Postprandial Lipemia (PPL)

The current school of thought believes that an acute bout of aerobic exercise is able to attenuate PPL. This recommendation comes with a wide range of intensities and times that exhibit change, ranging in intensity from 25-90% of maximal oxygen uptake (VO_{2max}) and in total time from 30-120 minutes (Beidleman et al., 2008; Gill & Hardman, 2000; Herd et al., 2001; Kim, Park, Trombold, & Coyle, 2014; Malkova et al., 2000; Miyashita, Burns, & Stensel, 2009; Trombold, Christmas, Machin, Kim, & Coyle, 2013; Zhang et al., 1998). On the other hand, there seems to be little in the way of literature as it relates to chronic training status or chronic exercise regimens and their relation to PPL. A study conducted by Holloszy et al. (1964) investigated the effects of a 6-month endurance training program, but PPL was not investigated in this study, only fasting lipids were looked at. Those studies that did investigate the relationship between these factors generally had subjects withhold from exercise performance to determine at what point the transient effect of exercise on blood triglyceride is abolished (Hardman, Lawrence, & Herd, 1998; Herd et al., 2000). Several factors have been proposed to affect the reduction in PPL via exercise: timing of exercise in relation to a high fat meal

(Beidleman et al., 2008; Zhang et al., 1998), especially previous bouts of exercise (Herd et al., 2000), intensity of exercise and whether exercise is continuous or intermittent (Kim et al., 2014; Miyashita et al., 2009; Trombold et al., 2013), and diet and exercise energy balance (Gill & Hardman, 2000; Kim et al., 2016).

2.3.2 Timing of Exercise on PPL

Understanding the point at which exercise begins to have an effect on postprandial triglyceride levels and the point at which that effect disappears is crucial in maintaining ideal triglyceride levels, especially if a diet is high in triglyceride. Zhang et al. (1998) looked at the postprandial responses in a group of trained men during a control without exercise, exercise after a high fat meal, exercise 1-hour prior to the high fat meal, and exercise 12-hours prior to the high fat meal. While the control had the highest triglyceride area under the curve (AUC), exercising after a meal had negligible effects on triglyceride AUC. However, both conditions with exercise prior to the meal demonstrated substantial reductions in triglyceride AUC, with 12-hours pre-meal producing similar reductions to 1-hour pre-meal (Zhang et al., 1998). This prior exercise phenomenon was also observed by Herd et al. (2001) when subjects exercised the afternoon prior to an oral fat-tolerance test. Later research was able to further validate that exercise prior to a high fat meal attenuates the postprandial triglyceride response generally seen. Silvestre et al. (2008) looked at exercise 4-hours and 16-hours prior to the meal and their relation to a non-exercising control condition. As previously demonstrated, both conditions significantly reduced triglyceride AUC as compared to the control, but were not significantly different from each other. This prior exercise effect may extend farther than 12-16 hours before the test meal. It has been suggested that postprandial triglyceride

response may be attenuated for at least 42-hours post-exercise and return to baseline by 60-hours post-exercise, regardless of prior training status (Herd et al., 2000; Kantor, Cullinane, Herbert, & Thompson, 1984) Other groups have suggested that this transient effect may only last 24-40 hours before disappearing (Maraki & Sidossis, 2013). The most common explanation for this delayed effect is an upregulation of LPL, which allows for greater clearance of blood triglycerides due to an increase in binding sites along the luminal wall (Herd et al., 2001; Herd et al., 2000; Kantor et al., 1984; Maraki & Sidossis, 2013). However, LPL levels have been noted to return to baseline 24-hours post-exercise (Seip & Semenkovich, 1998) or they may have not increased as a result of exercise at all (Maraki & Sidossis, 2013). Thus, the cause of exercise's effect on PPL is only partially elucidated through an LPL mechanism.

2.3.3 Exercise Intensity and Continuity

The continued popularity of interval style training (particularly high-intensity interval training [HIIT]) presents an important question regarding intensity and intermittency and their relationships with PPL. Trombold et al. (2013) found that incremental AUC for triglycerides was significantly lower for high-intensity interval style exercise than both isoenergetic moderate intensity continuous exercise or a non-exercise control. This effect was attributed to a higher rate of glycogen utilization during exercise, which subsequently increased the rate of postprandial fat oxidation, lowering postprandial triglyceride concentrations. This triglyceride lowering effect of intermittent high intensity exercise was also observed in the fasted state (Bellou et al., 2013). While this does not represent a potential postprandial lowering, the effect on fasting levels helps build the case for intense exercise to lower blood triglyceride concentrations. To achieve

lower postprandial triglyceride levels, it may not be necessary to do as great of an intensity or complete the total duration in a single bout. A series of six, 5-minute bouts of running at 70% VO_{2max} was shown to reduce postprandial triglyceride response as compared to a non-exercise control (Miyashita et al., 2009). However, it is important to note that these results did not investigate how intermittent exercise throughout the day compared to a single continuous bout of exercise (Miyashita et al., 2009). Another study conducted by Kim et al. (2014) furthered this notion that exercise may be done in an intermittent fashion and at a lower intensity. While the study found that a bout of moderate-intensity continuous running produced the greatest reduction in PPL, an accumulation of isoenergetic intermittent walking throughout the day still produced significant reductions in PPL as compared to a non-exercising control (Kim et al., 2014). This notion that PPL can be attenuated through low-intensity walking throughout the day suggests that a simple increase in non-exercise physical activity may be all that is necessary to improve blood lipid levels and subsequently improve cardiovascular health.

2.3.4 Exercise-Induced Energy Deficit

The impact of exercise induced energy deficit is another factor that has been explored to explain the reduction in PPL. Gill and Hardman (2000) investigated the relationships between exercise-induced and dietary-induced energy deficits on PPL as they observed prior research had not accounted for a relative energy deficit. This study found that, while an exercise-induced energy deficit (e.g. not replacing energy utilized during exercise via food) exhibited a significant reduction in PPL, a dietary-induced deficit did not. The results here suggested that some other mechanism was attributable to this PPL reduction (Gill & Hardman, 2000). The relationship between exercise-induced

energy deficit has been expanded upon with a notion that PPL reductions are only maintained when the energy deficit is not replaced by a meal (Burton, Malkova, Caslake, & Gill, 2008; Harrison et al., 2009). However, the abolishment of the PPL reductions with exercise-induced energy deficit may be related to the composition of the replacement meal. Both Burton et al. (2008) and Harrison et al. (2009) used either a moderate (~50%) or high (100%) carbohydrate replacement meal. Thus, the lack of difference found in PPL values may be due to carbohydrate replacement rather than something else. This notion of high and low carbohydrate replacement meals was investigated by Trombold et al. (2014) using a high carbohydrate content of ~83% and a low carbohydrate content of 12.5%. Although both replacement meals completely replaced energy lost via exercise, only the low carbohydrate meal maintained the reductions in PPL generally seen with exercise. The lack of PPL reduction in the moderate to high carbohydrate meals may be caused by a cascade of events stimulated by an influx of carbohydrate immediately post-exercise. Immediate intake of carbohydrate has been determined to be the most effective way to replenish muscle glycogen (Ivy et al., 2002; Ivy, Katz, Cutler, Sherman, & Coyle, 1988) especially when that carbohydrate is supplemented with protein (Ivy, Ding, Hwang, Cialdella-Kam, & Morrison, 2008). The amount of carbohydrate consumed in each of these tests may have been adequate to mostly or completely restore muscle glycogen levels (Costill et al., 1981) prior to a subsequent PPL test. However, if muscle glycogen levels have been supercompensated prior to complete carbohydrate uptake, insulin sensitivity will fall (Kuo, Browning, & Ivy, 1999), potentially leaving a large amount of carbohydrate in the blood to eventually be taken up elsewhere, particularly the liver. As mentioned before, the increase in carbohydrate in the liver may increase *de novo* lipogenesis, subsequently increasing VLDL secretion and PPL (Hellerstein, 1999; Nichols, 1969).

2.4 Potential Roles for Non-Exercise Physical Activity

Evidence has concluded that exercise is beneficial in improving postprandial triglyceride responses and endothelial function, which partially reduces the risk for developing cardiovascular disease. However, an increasing number of individuals are spending less time being physically active, let alone engaging in exercise, and more time being sedentary (Hansen, Kolle, Dyrstad, Holme, & Anderssen, 2012; Matthews et al., 2008). An increasing body of evidence is suggesting that the risk for mortality increases as time spent sedentary, especially continuous sedentary time, increases (Dunstan et al., 2010; Dunstan, Thorp, & Healy, 2011; Ekelund et al., 2016; Healy, Matthews, Dunstan, Winkler, & Owen, 2011; Katzmarzyk, Church, Craig, & Bouchard, 2009). However, the risks associated with increased sitting time may be reduced with adequate time spent in moderate-to-vigorous physical activity (MVPA) (Ekelund et al., 2016; Hamer, Stamatakis, & Steptoe, 2014; Howard et al., 2015; Katzmarzyk et al., 2009) or by breaking up sedentary time with adequate physical activity (Carter & Gladwell, 2016; Morishima et al., 2016; Thosar, Bielko, Mather, Johnston, & Wallace, 2015). To combat the increasing time spent sedentary, it is essential to identify effective ways to reduce the detrimental effects of sedentary time.

2.4.1 Impact of Sitting on Postprandial Triglycerides

Prolonged physical inactivity (e.g. prolonged sitting, bed rest, etc.) has been associated with insulin resistance, hypertriglyceridemia, ectopic fat storage (Bergouignan, Rudwill, Simon, & Blanc, 2011), and endothelial dysfunction (Morishima et al., 2016; Restaino et al., 2016). As it relates to prolonged sitting, PPL may be caused

by a reduction in muscular LPL activity. In a rat based model, heparin released LPL activity was significantly decreased, particularly in more oxidative muscle fibers, and triglyceride uptake was significantly decreased after a period of prolonged inactivity. These results suggest that muscle LPL is extremely sensitive to activity levels and may support the notion that a minimum level of physical activity must be reached to ensure adequate LPL activity and triglyceride uptake (Bey & Hamilton, 2003). These results were further supported by Zderic and Hamilton (2006) when they showed that inactivity suppresses LPL activity by ~90% in oxidative tissues. This reduction may be further exacerbated by an insulin resistance-mediated redistribution of energy towards VLDL production. As mentioned earlier, when insulin sensitivity falls, carbohydrate cannot be taken up into muscle and other tissue and will most likely be converted to VLDL in the liver (Hellerstein, 1999; Nichols, 1969). Olsen et al. (2008) were able to support the notion that increased physical inactivity leads to decreased insulin sensitivity and an increased triglyceride AUC.

2.4.2 Physical Activity's Ability to Attenuate PPL

Finding the optimal amount of physical activity, whether it be exercise or non-exercise (non-exercise activity thermogenesis; NEAT), is essential to reducing the incidence of cardiovascular disease through reductions in PPL driven atherosclerosis. A currently expanding field of research is beginning to suggest that physical activity and reductions in sitting time lead to reductions in metabolic and cardiovascular risk (Bey & Hamilton, 2003; Duvivier et al., 2013; Ekelund et al., 2016; Healy et al., 2008; Kim et al., 2016; Peddie et al., 2013). As it relates to PPL, physical activity may restore or prevent the loss of LPL functionality seen with sitting, which would prevent the accumulation of

triglycerides in the blood (Bey & Hamilton, 2003). Some studies are also suggesting that intermittent physical activity breaks may also be more effective at reducing PPL than a single bout of continuous exercise (Duvivier et al., 2013; Healy et al., 2008; Peddie et al., 2013). This improvement caused by regular breaks may also be mediated by improvements in glucose transport into cells (Peddie et al., 2013) or by increases in whole-body fat oxidation (Kim et al., 2016) that reduce the likelihood that endogenous triglyceride is produced and all sources of triglyceride are used for energy. However, some research is beginning to point out that current and emerging exercise recommendations (Ekelund et al., 2016; Garber et al., 2011) may not be enough to counteract the negative influences of sitting producing a so-called "exercise resistance" (Duvivier et al., 2013; Kim et al., 2016).

In a study conducted by Duvivier et al. (2013), subjects were subjected to one of three free living activity regimes: sitting for 14-hours/day, sitting for 13-hours/day with the 1-hour of sitting replaced with 1-hour of vigorous exercise, and a minimal-PA routine with 8-hours/day of sitting, 4-hours/day of walking, and 2-hours/day of standing. Of note was the relationship of energy expenditure between the groups. While the exercising group only sat for 1-hour fewer than the sitting group, the exercise considerably raised energy expenditure. In addition, while the time spent in activity and the intensity of activity was different between the exercise and minimal-PA groups, the energy expenditure was equivalent (Duvivier et al., 2013). Interestingly, the 1-hour bout of exercise was not able to improve blood triglycerides, cholesterol, or insulin sensitivity over sitting alone. However, minimal-PA was able to improve blood triglycerides and cholesterol over sitting and additionally insulin sensitivity over the exercise group (Duvivier et al., 2013). A later study looking at the effects of sitting on exercise's ability to normally lower PPL emphasized the role that sitting may play in health (Kim et al.,

2016). In this study, subjects underwent three trials of 5-days: sitting for >14-hours/day with a hypercaloric energy balance, sitting for >14-hours/day with a eucaloric energy balance, and active walking/standing with ~8-hours/day of sitting, with a eucaloric energy balance, relative to the extra energy expenditure. On day 3, subjects underwent a HFTT. Following each trial, subjects performed a 1-hour bout of running and another HFTT. Results showed that regardless of energy balance, sitting for >14-hours/day diminished the ability of exercise to attenuate PPL or increase fat oxidation when comparing the second HFTT to the first (Kim et al., 2016). Unfortunately, the study design did not include a control trial that did not perform the bout of exercise on day 4. This omission may have led to the inability for the bout of exercise to reduce any of the metrics measured during the HFTT. Had there been a control group that did not perform exercise, it may have been possible to observe a decline in PPL, albeit blunted, or to truly determine that prolonged sitting abolishes the generally beneficial effects of exercise. These studies highlight the negative consequences that prolonged sitting may have, regardless of exercise or energy balance. It is thus imperative that research begins to understand how physical activity and exercise may reverse the effects of sedentary time or if the population needs to spend less time being sedentary to improve cardiovascular and metabolic health.

2.5 SUMMARY

Atherosclerosis and other cardiovascular diseases present a serious problem towards general public health and their causes are continually being uncovered. From dyslipidemia (Mozaffarian et al., 2015; Nordestgaard et al., 2007; Zilversmit, 1979) to endothelial dysfunction (Anderson et al., 1995; Davignon & Ganz, 2004), researchers are

beginning to understand the mechanisms that play into impaired cardiovascular health. However, rather than prescribe pharmaceutical intervention, the proposed benefits of physical activity and exercise to reduce the incidence of cardiovascular disease by controlling blood lipids and endothelial dysfunction seem to be a more cost-effective and an ideal method to reversing a startling trend that affects roughly 600,000 Americans every year (National Center for Health Statistics, 2016). While the study conducted by Kim et al. (2016) is a good starting point, the lack of a control group limits the revelations it can show. It is thus necessary to follow-up with the results found there to determine whether the PPL response to exercise is truly blunted by sitting and further demonstrate that prolonged sitting has deleterious effects on metabolic and cardiovascular health. Continued and extensive research is needed to understand how sedentary behavior drastically influences cardiovascular risk factors and how the mechanisms behind sedentary time and physical activity/exercise interact.

Chapter 3: Methodology

3.1 Research Participants

Ten healthy, untrained to recreationally active men (n=5) and women (n=5) participated in this study. Subjects were allowed to participate in the study if they were apparently healthy, did not have a history of cardiovascular or metabolic problems, were not on medication that would directly affect lipid or carbohydrate metabolism, and were able to participate in extended durations of moderate to vigorous activity. The Institutional Review Board of the University of Texas at Austin approved the recruitment of participants and the protocols used in this study. Participants were given verbal and written descriptions of all protocols and measurements and informed of the purpose and potential risks involved with this study.

3.2 Research Protocol

All participants completed two different trials in a counterbalanced, crossover fashion, with each trial occurring over seven days with at least a week-long washout period between each trial. The first two days of each trial served as a control period (C1 and C2) that allowed for familiarization and control for activity and diet prior to the intervention. Following this, subjects completed four days (D1-D4) during which they sat for ~13.5 hours per day. On the evening of D4, the sitting with exercise group (SIT+EX) performed a 1-hour bout of exercise at ~65% VO_{2max} on a laboratory treadmill. On the morning of day five (D5), all subjects ingested a high fat shake (i.e. high fat tolerance tests; HFTT) and PPL responses were measured over the subsequent 6-hour period. A pictorial description is provided in Figure 1. Prior to partaking in any of the experimental conditions, subjects visited the Human Performance Laboratory (HPL) to undergo submaximal and maximal oxygen uptake (VO_{2max}) testing that was used to determine the

relative intensity of the exercise bout each participant performed on D4 in the SIT+EX group. In addition, subjects had their resting metabolic rate (RMR) measured at this time. During the initial visit, each subject had the study explained to them, signed an informed consent, and was able to ask any questions. Following the submaximal and maximal exercise testing, subjects were instructed on the proper way to use and attach the activity monitor.

During each trial, participants refrained from any exercise and kept physical activity to a minimum to achieve >13 hours per day of sitting. The diet followed was standard in macronutrient breakdown (US Department of Health and Human Services, 2011) and eucaloric such that subjects replaced the approximate number of calories needed as determined by RMR. Subjects in the SIT+EX group also consumed additional calories (via commercially available protein bars; Clif® Builder's®; approximately 40% carbohydrate, 30% fat, 30% protein) following the exercise bout to offset those lost during exercise. On the evening of D4, participants consumed a low-fat meal as fat in the previous meal can affect the response to a high-fat test meal (HFTM) (Fielding et al., 1996; Trombold et al., 2014) and the SIT+EX group performed their one-hour bout of exercise. On the morning of D5, participants went through an HFTT at ~0800 hours following a 12-hour fast. Prior to the HFTT, subjects arrived at 0700 hours and had weight measured, baseline expired gas measured, and a blood sampling catheter inserted. A resting blood sample was taken and then the HFTM was consumed in 5-minutes. Blood samples were then taken hourly for the next 6-hours. During the HFTT, expired gas was collected for determination of carbohydrate and lipid metabolism. Participants had been resting for at least 15-minutes in a relaxed, seated position, followed by 10-minutes of expired gas collection via meteorological balloons performed at 2, 4, and 6-hours post-HFTM ingestion. For the duration of each HFTT, participants sat quietly

reading, watching movies, and/or browsing the Internet. Participants were allowed to use the nearby restroom.

During C1 and C2, participants were asked to refrain from planned exercise, but achieve ~7,000 steps/day, corresponding to a "low level of physical activity" (Tudor-Locke & Bassett, 2004). On D1-D4 participants went through one of two conditions: prolonged sitting (~13.5 hours/day) without acute exercise (SIT) or prolonged sitting with 1-hour of acute exercise (SIT+EX). During the SIT+EX trial, the bout of exercise corresponded to 1-hour on a laboratory treadmill at ~65% VO_{2max}.

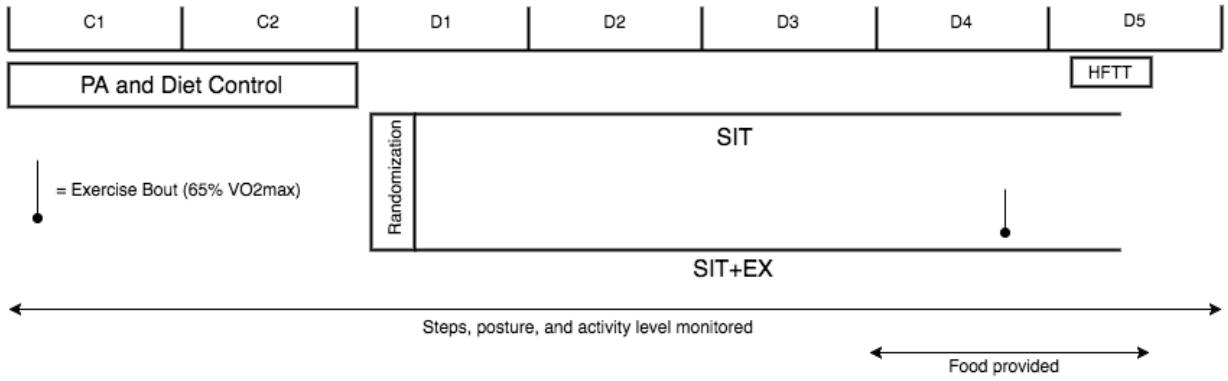

Figure 1: Pictorial representation of experimental design

3.3 MEASUREMENTS

3.3.1 Anthropometric Measurements

Subject body mass, as taken by a digital scale (Ohaus, CW-11, Parsippany, NJ). and recorded to the nearest 0.05 kg, was measured prior to any testing done at each visit. Height was measured using a standard stadiometer during the initial visit. Body mass and height were used to calculate BMI as a ratio of body mass to height squared.

3.3.2 Blood Sampling and Analysis

After 12-hours of overnight fasting, blood was collected during the HFTT via antecubital venous catheter. Approximately 4 mL of blood was taken for analysis at each draw during the HFTT at regular intervals (Pre, 1, 2, 3, 4, 5, and 6-hours post-meal). Prior to catheter insertion, the location of insertion was identified, cleaned, and sterilized thoroughly with an alcohol preparation wipe. After catheter insertion, a one-way valve and line was attached to allow for both blood collection and saline flush. When blood was not being drawn, a syringe filled with sterile normal saline was attached to the valve to allow for 1 mL of saline to be flushed through the line periodically (approximately every 5-10 minutes) to ensure patency. Catheters were inserted by a certified phlebotomist. After blood was transferred to K_2 EDTA tubes (BD Vacutainer, Fisher Scientific, Hampton, NH), the tube was then centrifuged at 3,000 revolutions per minute at 4°C for 15-minutes. Plasma was then aliquoted from the K_2 EDTA tube to an available Eppendorf tube labelled with identical subject information. The Eppendorf tubes were then stored at -80˚C in a locked laboratory freezer until later analysis. After blood samples were analyzed, they were discarded according to The University's Environmental Health and Safety guidelines. Plasma glucose was measured by a spectrophotometric method using commercially available kits (Pointe Scientific, Inc.

Canton, USA). Plasma triglyceride was also measured using a spectrophotometric method using commercially available kits (Pointe Scientific, Inc., Canton, USA). Appendix C contains detailed descriptions of each analysis performed.

3.3.3 Diet

Diet during each control period was kept constant and remained the same for each trial. During each experimental trial, diet was maintained at a eucaloric level as estimated from resting metabolic rate. Subjects were asked to achieve a diet that would provide a standard macronutrient breakdown (US Department of Health and Human Services, 2011). In addition, all food was logged using the free MyFitnessPal program/mobile application (MyFitnessPal, Inc.) and subjects were asked to consume the same food from Trial 1 in Trial 2. Additional energy expenditure from exercise sessions was estimated via indirect calorimetry using gas analyzers and was then replaced with additional food post-exercise. Food was provided for the subject on Day 4 to ensure strict adherence to a particular macronutrient profile, as certain meals in the 24-hours prior to HFTT can greatly impact postprandial triglyceride concentration, particularly the previous meal to the HFTM (Trombold et al., 2014). During the HFTT, the test meal was composed of portions of melted ice cream and half-and-half creamer to achieve a macronutrient and caloric profile based on body weight (carbohydrate = 1.12g/kg, fat = 1.20g/kg, protein = 0.35g/kg; total calories = 16.8kcal/kg).

3.3.4 Heart Rate

Heart rate was recorded and logged during exercise bouts via wearable heart rate monitor (HRM) (Polar Electro Inc., Lake Success, NY).

3.3.5 Maximal and Submaximal Exercise Tests

Submaximal exercise testing was conducted on a treadmill at 0% grade with 4 stages of increasing speed, with each stage lasting 5-minutes in duration. This served to determine oxygen uptake (VO_2) during moderate intensity exercise as well as approximate the intensity needed to reach 75% of VO_{2max} to be used as the starting point for subsequent VO_{2max} tests and 65% of VO_{2max} for the 1-hour bout of exercise. After 15-20 minutes of rest, participants completed a graded VO_{2max} test using a progressive treadmill protocol designed to last 8-12 minutes. Participants began at a speed that elicited 75% of VO_{2max} at 0% elevation for 4-minutes. At 4-minutes, the elevation was brought to 4%. At 6, 8, and 10-minutes, the elevation was brought to 6%, 8%, and 10%, respectively. If the subject was able to continue after this, the elevation was increased 2% every 1-minute until volitional exhaustion was reached. Gas analysis was performed using oxygen and carbon dioxide analyzers (Applied Electrochemistry, Models S-3A/I and CD-3A, respectively) while the subjects breathed through a one-way valve (Hans Rudolph, Kansas City, MO). Ventilation was measured via an inspiratory pneumotachometer (Hans Rudolph, Kansas City, MO). VO_{2max} was confirmed by at least 2 of the 3 following measures: a leveling off of oxygen uptake despite increasing intensity, HR within ±12 beats/min of age-predicted max (Predicted HR_{max} = 208 – 0.7*(age)), and a rating of perceived exertion (RPE) of 17 or higher out of 20 (Borg, 1982).

3.3.6 Physical Activity Monitoring and Step Monitoring

Levels of physical activity/inactivity were monitored almost continuously over the course of 6.5-days using a small, noninvasive monitor (activePALμ, PAL Technologies, Glasgow, Scotland). This activity monitor measured roughly 2in x 1in x 0.1in in size and was worn anteriorly on the thigh, roughly halfway between the inguinal

crease and the proximal border of the patella. The monitor was placed in a small rubber sheath and attached via transparent film dressing. The activity monitor was not waterproof and could not be worn while showering. Subjects were thus instructed to remove the device prior to showering and were provided with the materials to change the dressing immediately after showering once the area was dry. The monitor was attached on the evening prior to C1 and was only removed for 30-minutes each day to allow for time to shower. The monitor was used to make differentiations between a variety of activities, including supine/sitting, standing, and stepping. The same monitor was used to track step count. This device was able to determine all of this information through a combination of accelerometers and inclinometers. A further pedometer, either a standard pedometer or one accessed via smartphone, was used by the participants to use as visual feedback for daily step count as the normal monitor did not provide feedback until synced to a computer.

3.3.7 Postprandial Gas Exchange/Resting Metabolic Rate

Postprandial metabolic gas measurements and resting metabolic rate (RMR) gas measurements were performed via meteorological balloons. Participants were seated in a relaxed manner for 15-minutes, followed by gas collection for 10-minutes. During postprandial and RMR measurements, subjects breathed through a one-way valve (Hans Rudolph, Kansas City, MO) that was directly attached to a meteorological balloon. This gas sample was allowed to sit for 15-minutes before a 6-foot capillary sampling line was inserted via three-way stopcock to measure O_2, CO_2, and N_2 concentrations via mass spectrometer (Perkin-Elmer MGA 1100, St Louis, Missouri). Expired gas volume was then determined by connecting the meteorological balloon to a spirometer (Vacumed, Ventura, CA) and passing the air through.

3.4 STATISTICAL ANALYSIS

Incremental (AUC_i) and total area under the curve (AUC_t) for plasma concentrations of triglyceride (TG) and glucose were calculated using the trapezium rule. Once calculated, paired, two-tail t-tests were used to test for differences. Daily step count, caloric intake, and time spent in each posture/activity were analyzed using one-way ANOVA. Fasting and postprandial responses of respiratory exchange ratio (RER), fat oxidation, glucose, and TG were analyzed using two-way ANOVA with repeated measures (trials and time). Post-hoc Bonferroni corrections were performed to determine if statistical significance exists. All data were analyzed using GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA). All data are presented as means and standard error of the mean (SEM), unless otherwise noted. The level for statistical significance was set at $\alpha=0.05$

Chapter 4: Results

4.1 Subject Characteristics

Subject characteristics are outlined in Table 1. The total number of subjects was 10 (5 males, 5 females), with each subject completing both experimental trials. Subjects were mostly considered healthy, college age (23.8±4.0 years old) individuals that were untrained to recreationally active with a relative VO_{2max} of 43.43±10.02 $mL \cdot kg^{-1} \cdot min^{-1}$. Subject HR, $\%VO_{2max}$, and RPE during the one-hour bout of exercise were suggestive of moderate intensity and are shown in Table 1.

Table 1: Participant information (Body Mass Index, BMI; Resting Metabolic Rate RMR)

Variables	*Mean±SD*
Physical Characteristics	
Age (y)	23.8±4.0
Height (cm)	173.1±14.4
Body Mass (kg)	83.0±28.1
BMI ($kg \cdot m^{-2}$)	27.06±5.72
Resting Energy Metabolism	
RMR (kcal/day)	2302.1±694.9
Maximal Exercise Response	
VO_{2max} Absolute ($mL \cdot min^{-1}$)	3565.82±1260.24
VO_{2max} Relative ($mL \cdot kg^{-1} \cdot min^{-1}$)	43.43±10.03
Response to Exercise Bout	
Heart Rate (bpm)	158.14±10.98
Rating of Percieved Exertion	11.8±2.15
VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$)	27.45±6.96
$\%VO_{2\ max}$	63.07±5.18

4.2 Energy Intake

Energy intake for each day of both trials was recorded (Table 2 and Figure 2). For both control days (C1 and C2) and the first three days of the intervention (D1, D2, and D3), there was no difference in energy intake as subjects were asked to consume the exact same diet during Trial 2 that was consumed during Trial 1. By design, there was a significant interaction between trials (SIT and SIT+EX) and time (day) on D4 as the approximate caloric expenditure from exercise was fed to the subjects in addition to their normal provided meals ($p<0.001$). Post hoc analyses confirmed that the SIT+EX group consumed more calories than the SIT group on D4 ($p<0.001$). The added meal post-exercise was consumed with dinner immediately after the bout of exercise and was met via the use of Clif® Builder's® with the calories coming from approximately 40% from carbohydrate, 30% from fat, and 30% from protein. Energy intake during the HFTT also demonstrated no difference.

4.3 Daily Steps and Body Posture/Activity

Daily steps (Table 2 and Figure 3), as well as time spent sitting/supine, time spent standing, and time spent stepping (Table 2 and Figure 4) were recorded. A significant interaction was found between trials (SIT and SIT+EX) and time (day) for daily step count (Figure 3). Post hoc analyses determined that the SIT+EX group took significantly more steps than the SIT group on D4. There were no differences between the SIT and SIT+EX groups for time spent sitting (Figure 4A) or time spent standing (Figure 4B). However, there was a significant interaction between treatment (SIT and SIT+EX) and time (day) for time spent stepping ($p<0.001$). Post hoc analyses determined that on D4, the SIT+EX group stepped significantly more times than the SIT group ($p<0.001$). These differences were caused by the one-hour bout of exercise performed on D4.

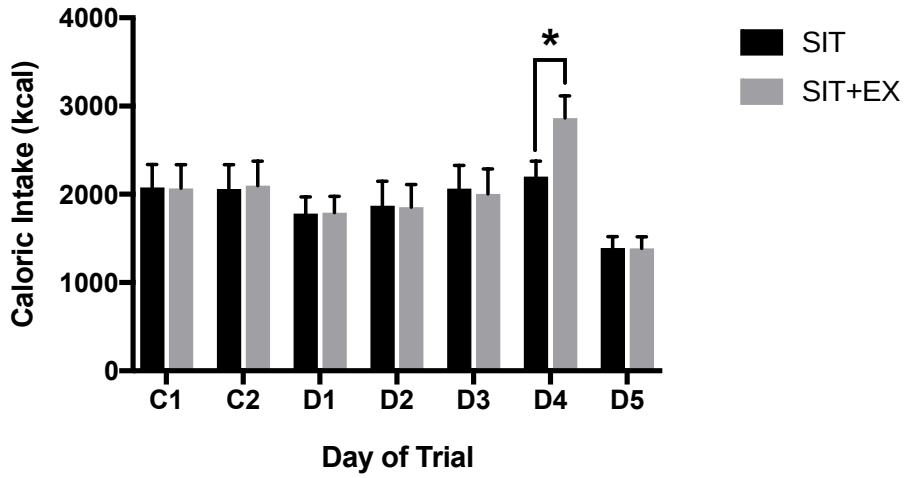

Figure 2: Average daily caloric intake (kcal/day) for both trials shown above. Control days (C1 and C2), intervention days (D1, D2, D3, and D4), and HFTT (D5) all represented. *Significantly different interaction between trials ($p<0.001$)

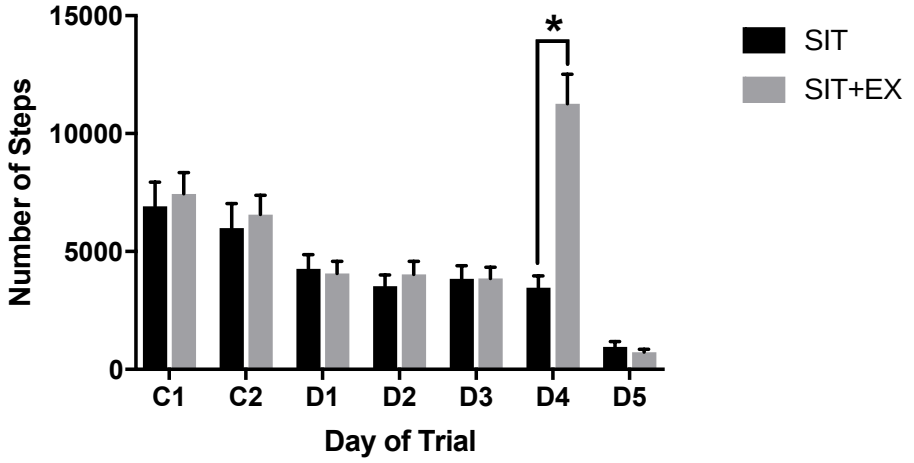

Figure 3: Average daily step count (steps/day) for both trials shown above. Control days (C1 and C2), intervention days (D1, D2, D3, and D4), and HFTT (D5) all represented. *Significantly different interaction between trials ($p<0.001$)

Table 2: Average daily steps, distribution of activity/posture, and caloric intake for both trials. *Significantly different from SIT ($p<0.001$)

Trial	Day of Trial						
	C1	C2	D1	D2	D3	D4	D5
Daily Steps (steps/day)							
SIT	6922.2±1019.9	5997.4±1031.9	4267.2±598.2	3528.0±477.8	3847.6±544.5	3466.8±503.6	955.00±232.10
SIT+EX	7438.9±899.6	6570.7±815.9	4073.3±510.2	4031.7±543.7	3850.9±476.9	11272.1±1242.6*	729.50±122.41
Distribution of Activity and Posture (min/day)							
Sitting/Supine							
SIT	1207.13±27.81	1248.73±43.02	1230.93±52.35	1293.33±17.94	1262.60±23.95	1293.40±25.39	619.52±65.46
SIT+EX	1173.93±46.06	1162.07±43.67	1241.27±38.94	1273.73±21.16	1219.67±25.71	1224.07±32.46	781.20±87.77
Standing							
SIT	142.00±19.49	117.80±30.52	154.87±46.56	104.07±14.25	127.47±19.41	111.07±21.22	30.93±7.72
SIT+EX	190.33±31.70	193.2±38.72	147.67±35.63	113.33±16.16	175.4±25.08	115.00±21.62	26.73±6.20
Stepping							
SIT	90.13±11.59	73.47±15.02	54.13±7.57	42.67±6.30	49.87±6.46	35.47±5.26	11.00±2.43
SIT+EX	75.73±17.19	84.80±9.51	51.00±6.62	53.00±6.89	45.00±6.96	100.93±13.47*	8.87±1.48
Caloric Intake (kcal/day)							
SIT	2079.00±259.88	2062.20±271.25	1780.80±189.48	1873.30±276.63	2065.30±264.63	2200.50±175.78	1391.36±131.61
SIT+EX	2068.30±265.80	2098.90±275.72	1791.50±185.62	1855.40±257.50	2006.10±282.00	2865.3±248.48*	1388.73±129.70

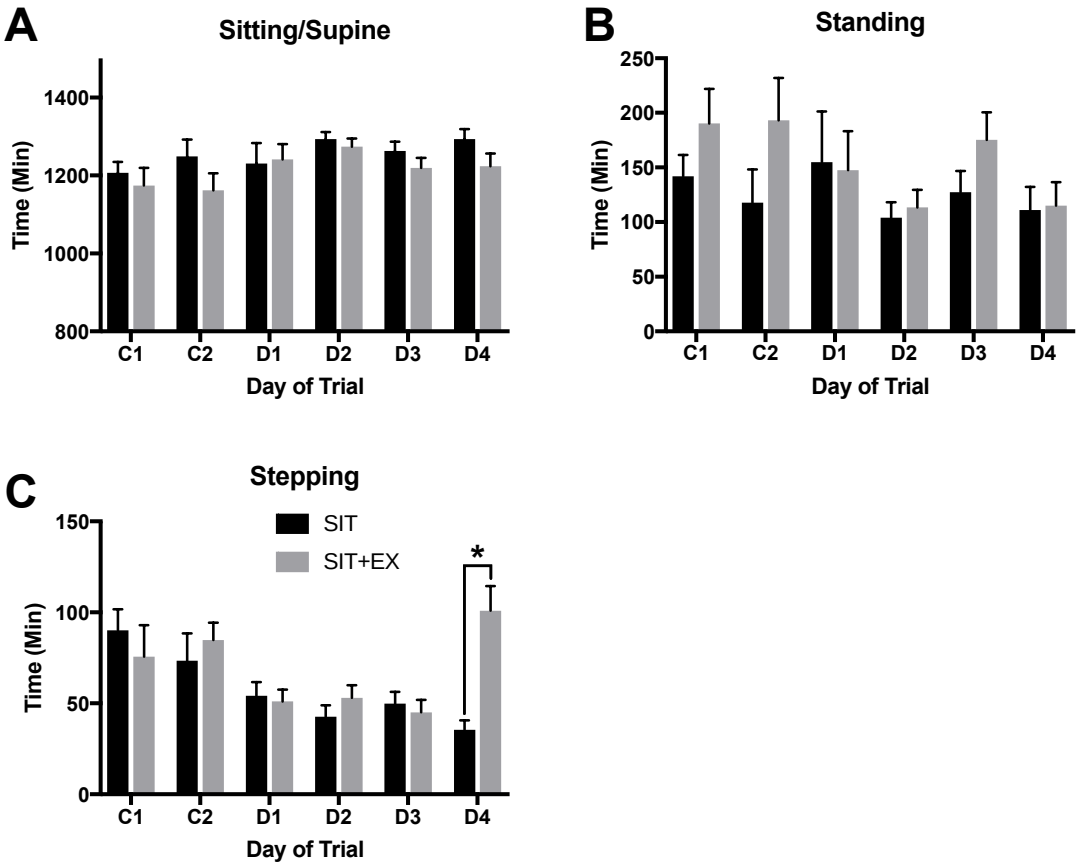

Figure 4: Time spent sitting/supine (A), time spent standing (B), and time spent stepping (C) are represented above. *Significantly different interaction between trials ($p<0.001$)

4.4 Postprandial Substrate Oxidation

Postprandial substrate oxidation was determined using indirect calorimetry (Lusk, 1924) (Table 3 and Figure 5). Oxidation calculations were limited to five paired subjects due to apparent hyperventilation at rest as defined by a respiratory exchange ratio (RER) greater than 1.00. RER was not found to be different between trials (Figure 5A). Percent carbohydrate oxidation (Figure 5B) and percent fat oxidation (Figure 5C) were found to have no difference between trials at any time point postprandial. Finally, no difference was found in postprandial energy expenditure (Figure 5D). While no difference was

found between trials, significant temporal differences were observed within trials (Table 3).

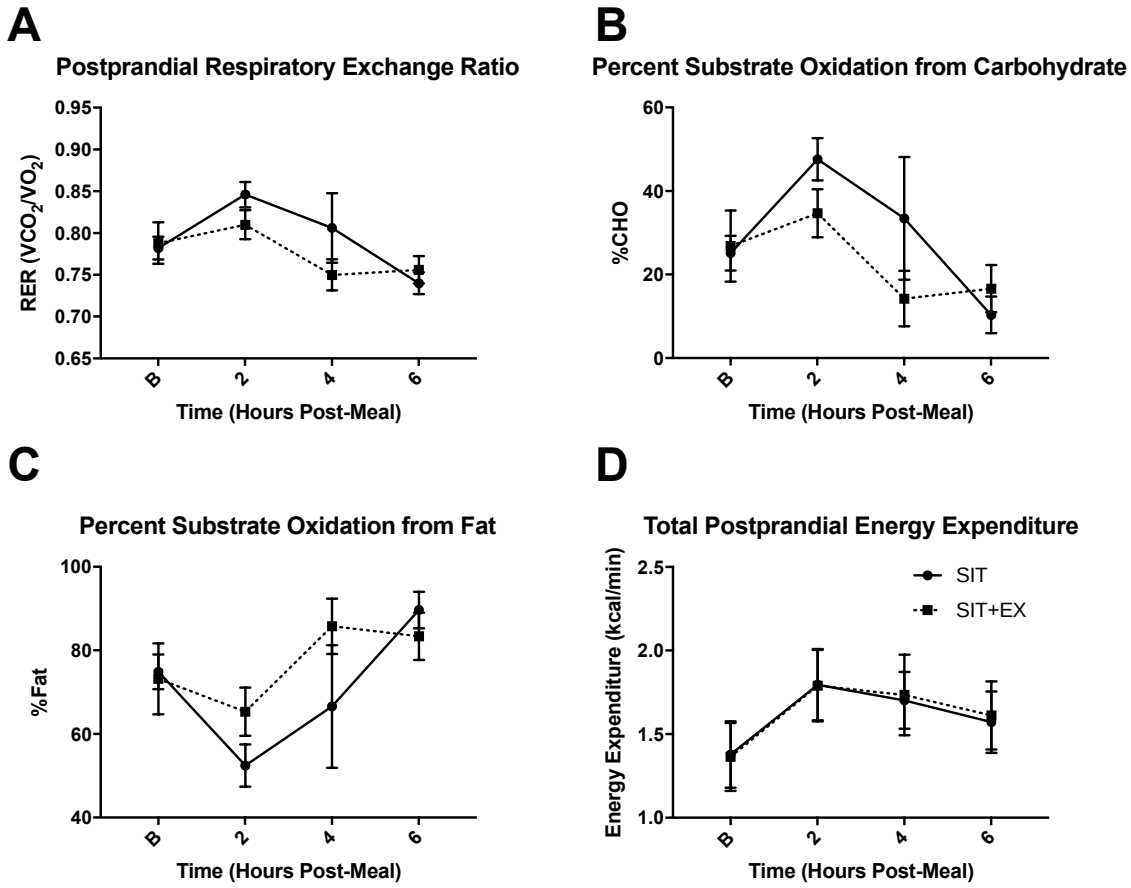

Figure 5: Postprandial substrate oxidation during HFTT. RER (A), percent carbohydrate oxidation (B), percent fat oxidation (C), and energy expenditure (D) are shown above. No significant differences were found at any time point between trials.

Table 3: Average values for postprandial substrate oxidation variables for duration of HFTT. (&) Significantly different from Baseline ($p<0.05$), (#) significantly different from Baseline ($p<0.01$), (*) significantly different from Hour 2 ($p<0.01$)

	Trial	
Measurement Point Postprandial	SIT	SIT+EX
Respiratory Exchange Ratio		
Baseline	0.781±0.012	0.786±0.024
Hour 2	0.846±0.015	0.809±0.017
Hour 4	0.805±0.043	0.749±0.019
Hour 6	0.737±0.013*	0.756±0.017
Percent CHO Oxidation		
Baseline	25.13±4.16	26.83±8.50
Hour 2	47.58±5.04	34.68±5.77
Hour 4	33.45±14.66	14.24±6.63
Hour 6	10.35±4.36*	16.62±5.66
Percent Fat Oxidation		
Baseline	74.88±4.16	73.17±8.50
Hour 2	52.42±5.04	65.32±5.77
Hour 4	66.55±14.66	85.76±6.63
Hour 6	89.65±4.36*	83.38±5.66
Energy Expenditure (kcal/min)		
Baseline	1.378±0.199	1.364±0.203
Hour 2	1.796±0.213#	1.790±0.213#
Hour 4	1.702±0.170&	1.734±0.241#
Hour 6	1.572±0.183	1.572±0.204

4.5 Plasma Triglyceride Concentrations

Plasma triglyceride concentrations were analyzed at each measured time point in both trials and as the incremental area under the curve (AUC_i) and total area under the curve (AUC_t). No significant difference was found between trials at any time point for the triglyceride curve. Individual time points within each trial were significantly different, however, when compared to either Baseline or Hour 1 (Table 4 and Figure 6A).

In addition, neither AUC_i nor AUC_t demonstrated a significant difference between trials (Table 5 and Figure 7).

4.6 Plasma Glucose Concentrations

Plasma glucose concentrations were analyzed at each measured time point in both trials and as the incremental area under the curve (AUC_i) and total area under the curve (AUC_t). As with the triglyceride concentrations, no significant differences were found at any time point between trials. However, significant differences were found at different time points within each trial's HFTT (Table 4 and Figure 6B). Furthermore, neither AUC_i nor AUC_t demonstrated a significant difference between trials (Table 5 and Figure 8).

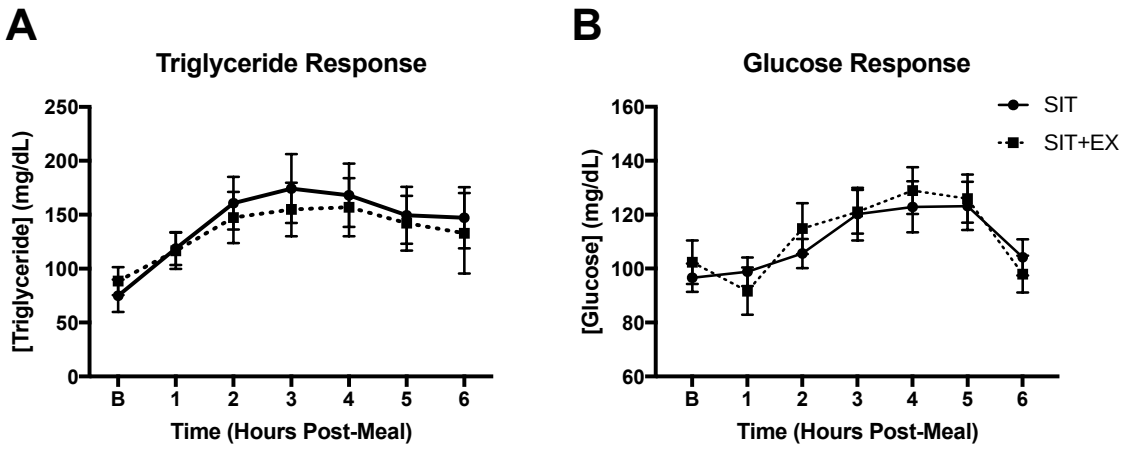

Figure 6: Plasma concentration response for triglyceride (A) and glucose (B) during the HFTT for both trials. No significance was found between trials in either measurement ($p>0.05$).

Table 4: Postprandial responses for plasma triglyceride and glucose concentrations. No significance was found between trials at any time point. Significance across time points within a trial are as follows: (*) $p<0.05$ compared to Baseline, (%) $p<0.01$ compared to Baseline, (#) $p<0.001$ compared to Baseline, ($) $p<0.05$ compared to H1, (^) $p<0.01$ compared to H1, (&) $p<0.001$ compared to H1, (+) $p<0.01$ compared to H4, and (?) $p<0.05$ compared to H5.

Trial	Day of Trial						
	Baseline	H1	H2	H3	H4	H5	H6
[Triglyceride] (mg/dL)							
SIT	74.87±15.11	118.71±15.26*	160.65±24.53$^{\#\$}$	174.28±31.98$^{\#\&}$	167.99±29.34$^{\#\^{}}$	149.43±26.48$^{\#}$	147.12±28.30$^{\#}$
SIT+EX	88.41±13.01	116.65±16.92	147.40±23.71$^{\#}$	154.79±24.87$^{\#\$}$	157.05±26.89$^{\#\$}$	142.26±25.58$^{\#}$	132.76±37.30$^{\%}$
[Glucose] (mg/dL)							
SIT	96.62±5.23	98.85±5.29	105.60±9.46	120.23±9.75	122.92±9.50$^{\%}$	123.23±8.98$^{\%}$	104.20±6.71
SIT+EX	102.38±8.06	91.65±8.77	114.86±9.46	121.07±8.13$^{\^{}}$	128.96±8.67$^{*\&}$	125.93±8.93$^{\^{}}$	97.95±6.86$^{+?}$

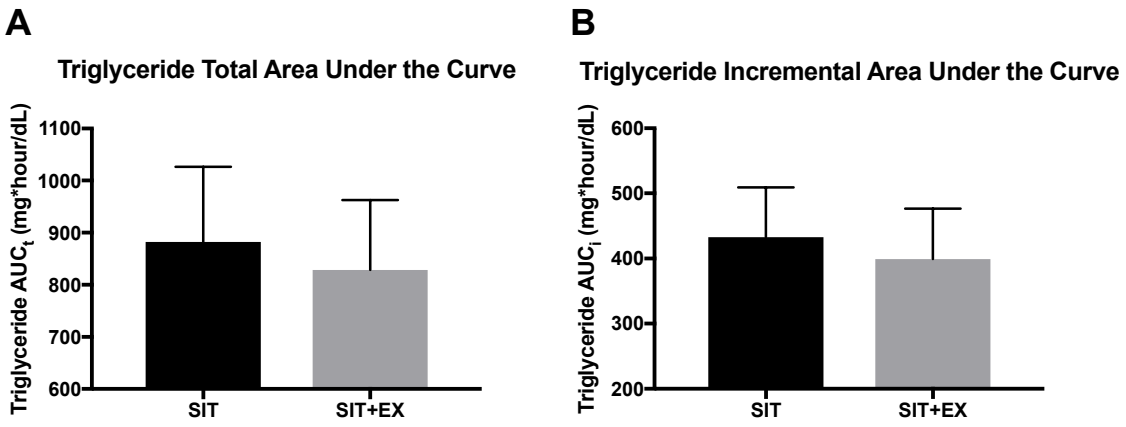

Figure 7: Triglyceride AUC_t (A) and AUC_i (B) for both trials. No significant difference was found between trials in either measurement ($p>0.05$).

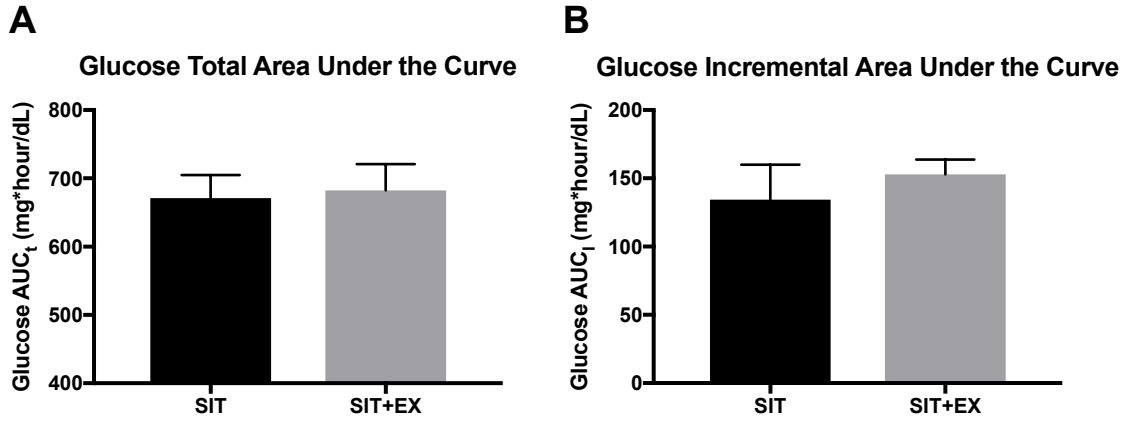

Figure 8: Glucose AUC_t (A) and AUC_i (B) for both trials. No significant difference was found between trials in either measurement ($p>0.05$).

Table 5: Incremental and Total areas under the curve for both triglyceride and glucose responses during HFTT. No significant differences were found between trials.

Trial	AUC_i	AUC_t
Triglycerides		
SIT	432.9±76.3	882.1±144.2
SIT+EX	399.3±77.1	828.7±134.2
Glucose		
SIT	134.5±25.35	671.2±33.49
SIT+EX	152.9±10.85	682.6±38.33

Chapter 5: Discussion

The main purpose of this study was to investigate the effect of several days of prolonged sitting on the ability of an acute bout of exercise to reduce PPL. In order to assess this interaction, the study had two, one week-long interventions that had participants perform four days of prolonged sitting (~13.5 hours/day) with one intervention containing a one-hour bout of moderate intensity (~63% VO_{2max}) exercise. The main hypothesis was that there would be no difference in the PPL response to a HFTT after the fourth day, despite the SIT+EX group receiving the one-hour bout of exercise. As hypothesized, the study was able to demonstrate that metabolic outcomes (e.g. plasma triglyceride and glucose concentration) were no different in the SIT+EX as compared to the SIT group, suggesting that the prolonged sitting abolished the ability of the acute bout of exercise to reduce PPL response.

Although it has been well documented that exercise is effective at lowering PPL and plasma glucose (Beidleman et al., 2008; Herd et al., 2001; Malkova et al., 2000; Zhang et al., 1998), more recent research (Duvivier et al., 2013; Kim et al., 2016) has shown that prolonged sitting or sedentary activity may invoke a type/form of "exercise resistance." While both Duvivier et al. (2013) and Kim et al. (2016) demonstrated this relationship between prolonged sitting and PPL, both studies exhibited missing elements of their design. The study by Duvivier et al. (2013) was limited to the examination of the effect of an oral glucose tolerance test after prolonged sitting, prolonged sitting with exercise, and physical activity throughout the day. While glucose tolerance is important, as it relates to atherosclerosis, triglyceride tolerance would be a better metric as postprandial triglyceride tolerance is more highly related to risk of cardiovascular disease (Bansal et al., 2007; Nordestgaard et al., 2007). While Kim et al. (2016) did look at

triglyceride tolerance, all three groups that were investigated performed the bout of exercise. The results thus focused less on the effect of the bout of exercise and more on the dietary differences and activity differences. This could be seen as a limitation to the design as there was effectively no control group used to investigate the effects of a one-hour bout of acute exercise. The current study was subsequently modeled after the Kim et al. (2016) study, but with only two groups. In using only two groups, one of them being a control that did not exercise (SIT), we could isolate the effects of exercise as everything else was kept controlled between trials. This extended to the fact that caloric intake in both groups was maintained close to energy balance, particularly D4, when subjects in SIT+EX were fed an average of 650 kilocalories.

In the present study, the goal was to emulate conditions that would be common in real world scenarios. Much like the Kim et al (2016) study, subjects were seated or supine for the vast majority of their intervention days. While the activity monitor could not distinguish between seated and supine time and subsequently sleeping time, under the assumption of an average 7-8 hours of sleep per night, subjects were still seated for over half their day and the vast majority of their waking hours. This extended time spent sitting helps connect these results to real world application as sitting is the predominant form of physical inactivity (Hamilton, Hamilton, & Zderic, 2007). In addition, subjects averaged <4,000 steps/day during each intervention outside of the 1-hour bout of exercise, which would be indicative of a sedentary lifestyle (Tudor-Locke & Bassett, 2004), beyond the time spent sitting. Given that no differences were found between daily steps, body posture/activity, or caloric intake, except for the day of the exercise bout, this level of sedentary behavior was maintained in both trials.

When looking at triglyceride and glucose responses, it was important to look at the temporal responses to see how PPL changed over time, but also at the area under the

curve (AUC) to quantify the overall response to the high fat test meal. While a difference was not seen between trials for the temporal response in plasma triglyceride or glucose concentrations, there was a significant difference found at different time points with each trial. This within trial response was to be expected, however, as the absorption of glucose and triglyceride is a delayed response. Plasma glucose generally peaks soon after ingestion and begins to fall off after that while plasma triglyceride generally takes about 2-hours post-ingestion to begin increasing, eventually peaking about 3-4 hours post-ingestion. While plasma triglyceride followed the trend that would be expected, the average plasma glucose took slightly longer to peak and remained high through ~4-hours postprandial. Two reasons may help explain this observation. The first is that the wavelength filter used in the microplate reader for plasma assay analysis had a relatively wide range, which may have reduced the accuracy of our measured concentrations of glucose. The second reason may be that the introduction of high levels of fat in the diet may reduce the glycemic response to a high carbohydrate meal (Jenkins et al., 1981), delaying the peak, and it may reduce the uptake of circulating glucose (Boden & Chen, 1995), extending the time plasma glucose stays elevated. However, as the study was set up in a crossover fashion and both trials underwent the HFTT, the difference in metabolism should have been derived from the exercise bout alone, if any difference existed. Furthermore, the plasma glucose results in the study by Kim et al. (2016) also exhibited this prolonged response.

Looking at the AUC for both triglyceride and glucose response, the PPL response can be better evaluated. As noted previously, no significant difference was found between trials in AUC_t for either triglyceride or glucose. This would suggest that sedentary behavior not only abolished the normal effects of exercise to reduce PPL not only across the test, but via the overall response as well. However, it has been suggested the AUC_t is

not the most appropriate test as it does not accurately reflect the rise in PPL (Carstensen, Thomsen, & Hermansen, 2003). Carstensen et al. (2003) suggests that AUC_i should be used instead as it has a stronger relationship with the postprandial triglyceride response and accounts for basal differences. However, when we examined the AUC_i for both triglyceride and glucose, we still found no significant difference between trials. The lack of difference between trials for glucose and triglyceride in either AUC_t or AUC_i continues to suggest that prolonged sitting and sedentary behavior significantly affect triglyceride and glucose metabolism. As it relates to cardiovascular disease, the impaired triglyceride metabolism seen after prolonged sitting may lengthen the amount of time atherogenic byproducts are in contact with the vasculature. This increased exposure to these byproducts may be increasing the rate of atherogenesis and subsequent cardiovascular problems as has been previously elucidated (Assmann et al., 1996; Jeppesen et al., 1998; Manninen et al., 1992; Zilversmit, 1979).

Substrate oxidation as calculated by indirect calorimetry might be able to show improved metabolic outcomes despite no difference in plasma profiles of triglycerides or glucose. If caloric expenditure were to remain the same from trial to trial, but SIT+EX were to demonstrate a higher percentage of energy expenditure from fat, then more of the circulating lipid might be oxidized. However, indirect calorimetry did not show a significant difference between RER, percent substrate from carbohydrate, percent substrate from fat, or total energy expenditure. This would suggest that beyond the effects that prolonged sitting has on circulating triglyceride and glucose, this sedentary behavior also has negative effects on substrate oxidation. However, the lack of a difference in gas analysis may be due to the small sample size. Perhaps due to the lack of experience with or comfort with the mouthpiece and one-way valve that was used to collect expired gas samples, three subjects consistently hyperventilated (as indicated by an RER over 1.00)

in both trials while two more subjects hyperventilated in one trial. This limited the number of subjects that could be analyzed for indirect calorimetry and may have reduced the ability to detect a difference between trials.

Although this study demonstrated that prolonged sitting, even with a one-hour bout of moderate exercise, has detrimental effects on triglyceride and glucose clearance, it did not directly investigate the mechanisms for exercise resistance and requires speculation. Lipoprotein lipase (LPL), particularly at the muscular level, is perhaps one of the first explanations for the impaired triglyceride clearance that has been observed. LPL has been observed to upregulate post-exercise for ~24-hours as extra binding sites appear along the luminal wall of the vasculature (Herd et al., 2001; Herd et al., 2000; Kantor et al., 1984; Maraki & Sidossis, 2013). This would generally be significant as LPL hydrolyzes the triglyceride attached to chylomicron and VLDL carriers to allow for uptake into the adjacent cells (Ginsberg et al., 2005) and is the rate-limiting enzyme for removing chylomicrons and VLDL from circulation (Wang & Eckel, 2009). However, due to the lack of difference in postprandial plasma triglyceride concentration found in the present study, it may suggest that prolonged sitting impaired or prevented the upregulation of LPL post-exercise. In fact, studies have shown prolonged inactivity decreases the amount of heparin released LPL and may reduce its activity by up to 90% (Bey & Hamilton, 2003; Zderic & Hamilton, 2006). LPL activity may not be the only cause of impaired PPL response.

Insulin sensitivity may be another mediator for impaired PPL response. It has been suggested that a reduction in daily activity may cause a decrease in insulin sensitivity (Olsen et al., 2008). This may be particularly pronounced in the disused muscles, potentially suggesting that a lack of muscular activity may be the cause for this reduction in insulin sensitivity (Krogh-Madsen et al., 2010). This relationship between

insulin resistance and dyslipidemia seems relatively linear as well, suggesting that dyslipidemia becomes more pronounced with an increase in insulin resistance (Reaven, 1993). These findings point towards an increase in dyslipidemia as a result of the physical inactivity derived insulin resistance. This relationship may occur due to the subsequent increase in circulating glucose. As the tissues, particularly muscle, are unable to take up glucose as effectively, the circulating glucose is more likely to be picked up in the liver and, via *de novo* lipogenesis, are converted to VLDL (Hellerstein, 1999; Nichols, 1969). This conversion from carbohydrate to lipid would greatly increase the PPL response. Furthermore, given the high concentration of carbohydrate used in the test meal, that may have been enough to stimulate this lipogenesis. However, rather than impaired glucose uptake influencing triglyceride uptake, other research suggests that the increase in circulating lipid actually affects glucose uptake (Boden & Chen, 1995). This suggests the possibility that the impaired PPL response is in part caused by the concomitant rise in glucose due to insulin resistance or that insulin resistance is imbued by the hyperlipemic response to the test meal. It is difficult to discern the difference in this study at the time, however, as insulin is not reported in this thesis. However, it might be postulated that the response to insulin secretion was similar as the plasma glucose response was similar between trials in response to a comparable meal.

While the present study helped answer the missing part of the design from the Kim et al. (2016) study, it is not without its limitations. First the sample size may not have been large enough to detect appreciable differences in our dependent measures. However, three more subjects were used in the present study than in the Kim et al. (2016) study, which may have improved our chances to find significant differences. Secondly, activity was not as controlled as it could possibly be, but to do so would require removing each individual from their normal routine and have them spend the duration of each week

in the laboratory. Doing so is highly unrealistic for the scope of this project, but it may be important in future studies to ensure adequate control over the subjects' lifestyles. Finally, the feeding back of the calories expended during the bout of exercise may have been the cause of the impaired PPL response in the SIT+EX as the replacement of energy lost has been reported to attenuate the exercise induced PPL reduction (Burton et al., 2008; Harrison et al., 2009), especially when the meal is high in carbohydrate (Trombold et al., 2014). In this study, however, the percentage of carbohydrate in the replacement meal was moderate (~40% energy from carbohydrate) and perhaps lower than the percentage of carbohydrate in a standard diet (US Department of Health and Human Services, 2011) In addition, the extra caloric intake resulting in a eucaloric balance post-exercise on D4 has been shown to produce a similar PPL response to a condition in which the exercise causes hypocaloric balance (Kim et al., 2016). Thus, the extra caloric intake on D4 of the SIT+EX trial most likely did not contribute to the impaired PPL response observed.

In conclusion, the data in the present study points to the notion that four days of prolonged sitting has detrimental effects on normal, exercise-induced metabolic responses. Rather than the reduction in triglyceride and glucose responses that would be expected after a one-hour bout of acute exercise, the results demonstrated no difference compared to the control group. The findings in this study suggest that sitting generates a resistance to exercise that cannot simply be overcome by an acute exercise bout. This highlights the need to look at several bouts of exercise, perhaps those that meet current exercise recommendations (Garber et al., 2011) or proposed non-exercise physical activity recommendations (Ekelund et al., 2016). The findings in this study coupled with those by Kim et al. (2016) emphasize the detrimental consequences prolonged sitting has

on human cardiometabolic health and underscores the need for further research to determine the best course of action of avoid the negative impact of prolonged sitting.

Appendix A: Informed Consent

Consent for Participation in Research

The Effect of Sitting and Moderate Exercise on Plasma Triglyceride Elevation After a Meal

Introduction

The purpose of this form is to provide you information that may affect your decision as to whether or not to participate in this research study. The person performing the research will answer any of your questions. Read the information below and ask any questions you might have before deciding whether or not to take part. If you decide to be involved in this study, this form will be used to record your consent.

Purpose of the Study

The purpose of this study is to investigate the effect of 4 days of sitting, and moderate exercise on plasma triglyceride elevation after a meal.

What will you be asked to do?

Before you can be admitted to the study, you will be given brief preliminary tests. This will include filling out a brief Health Research Questionnaire, and taking measurements of your height and weight. Only if you are apparently healthy and at low risk for cardiovascular disease will you be invited to participate in this study. Prior to your enrollment in the study, your maximal oxygen uptake (VO_{2max}) will be determined while running on a treadmill and also your heart rate during submaximal running will be determined.

Each trial will require seven days, with periodic visits to the HPL:

Trial 1: Plasma triglyceride responses with four days of mostly prolonged sitting.

Trial 2: Plasma triglyceride responses with four days of mostly prolonged sitting and a single one-hour bout of exercise on the night of the fourth day.

The order of protocols will be randomized.

Step-by-Step Protocol:

Week prior to the initiation*: Health history questionnaires, familiarization, VO_{2max} test.*

1. Arrival at the Human Performance Laboratory (HPL), informed consent, health history questionnaires, body mass and height.
2. Installation of the activity monitor.
3. Perform resting gas measurement.
4. Warm up for 5 minutes on a treadmill.
5. Perform submaximal exercise test with four treadmill speeds lasting five minutes each. The intensity will approximate 40, 60, 70 and 80% of age predicted maximal heart rate.
6. Recover for ~ 15-20 minutes (re-hydrate to pre-exercise bodyweight)
7. Perform continuous maximal oxygen consumption test; (VO_{2max}test, 8-12 min.)

■ ***Total time: 150 minutes***

Trial sessions

Control Phase: Control Day 1 and 2 (C1 and C2)

Day prior to Control day 1: Activity monitor installation

1. Arrival at the laboratory any time before 17:00 h.
2. Installation of activity monitor

- ***Total time: 30 minutes***

■ ***Total time spent during Control Phase: 30 minutes***

Intervention Phase: D1 - D4

Day 1-3: Sitting

For a both interventions, Sitting and step number (~2,000 steps)

1. Sitting in preferred place (>12 hours, not necessarily in HPL).
2. Arrival at HPL at 17:00 h on D3.
3. Meals for D4 provided in the laboratory.

- ***Total time: 30 minutes***

Day 4: Sitting, or Sitting & 1-hr treadmill running

For SIT, sitting and step number (~2,000 steps)

1. Sitting in preferred place (>12 hours, not necessarily in HPL).

2. Arrival at HPL at 17:00 h.
3. Dinner provided in the laboratory

For SIT- EX, sitting and a single bout of 1-hour exercise at 65%

1. Lying or sitting in their preferred place (>12 hours).
2. Arrival at HPL at 16:50 h.
3. Exercise at 65% VO_{2max} for one hour at around 17:00 h
4. Dinner provided in the laboratory.

- ***Total time: SIT- 30 min; SIT-EX- 120 min***

Day 5: High fat tolerance test and resting fat oxidation

1. Arrival at HPL, body weight, 8:00 h.
2. Catheter insertion and fasting blood collection.
3. High fat shake intake
4. Postprandial blood sampling hourly for 6 h (6 additional samples)
5. Expired gas collection for 20 minutes at 1, 3, 5 h after high fat shake intake
6. Detachment of the activity monitor

- ***Total time: 420 minutes***

■ ***Total time spent during Intervention Phase:***

- SIT: 480 minutes

- SIT+EX: 570 minutes

Total time per subject for both trials is approximately 1,050 minutes

What are the risks involved in this study?

None of the above procedures are expected to be unduly painful or unsafe in healthy individuals. The maximal oxygen uptake (VO_{2max}) and submaximal tests and 1 hour of moderate exercise at 65% VO_{2max} will be performed at 20 - 25ºC and relative humidity of ~ 50%. During VO_{2max} test, only the final 2 to 4 minutes of the test is at or near maximal levels of exertion and thus accompanied by a sensation of leg fatigue and heavy breathing. This moderate feeling of fatigue will subside soon after completion (i.e.; 2-10 min). There is a very small risk that participants could experience a muscular injury, such as muscle strain. It is also possible that muscle soreness may develop 24 to 48 hours after any given testing session. To help reduce these risks, a warm up session will be mandatory prior to performing these tests.

Blood samples will be drawn during each HFTT via venous catheter in the forearm or antecubital vein. A certified phlebotomist will insert the catheters. Minor discomfort may occur during the insertion of the catheter. The discomfort associated with the insertion of the catheter is similar to a venipuncture. Risks associated with placement of the catheter include bleeding, pain, swelling, bruising, infection, and thrombophlebitis. Approximately 42 ml of blood will be drawn per trial. Over the course of the entire study approximately 84 ml of blood will be drawn. This sample volume is approximately 6 tablespoons and <1.5% of the individual's total blood volume. If participants are found to have abnormally high triglyceride levels, they will be alerted of this and advised to follow-up with their primary care physician.

The activity monitor will be instrumented the day before each trial after taking a shower and will be equipped for 96 hours. Participants may experience a low-level annoyance from the wires touching the skin during the 48hr monitoring based upon previous practices in the lab. The activity monitor will be detached for 1 hour to load data to a computer, during which participants will take a shower on day 4 (the intervention day). Thus, participants will not be allowed to take a shower when the activity monitor is attached to the participants. A pedometer will be instrumented for a week before the initiation of the first trial for the estimation of an average daily step and throughout the trials. It will not give participants any discomfort as many use such instrumentation regularly.

The risk of any sort of cardiovascular complication in apparently healthy (no documented CV disease) individuals is very low, with no complications in 380,000 tests. Furthermore, in over 32 years involving more than 50,000 exercise sessions, no subject in the Human Performance Lab has experienced any cardiac event. The laboratory is currently equipped with AED. A CPR certified member of the research team will be present during all testing visits in the unlikely event of an adverse reaction.

During the tests participants may stop performing the task at any time and for any reason if he or she feels the need to do so. If participants wish to discuss the information above or any other risks participants may experience, participants may ask questions now or call the Principal Investigators.

What are the possible benefits of this study?

You will receive no direct benefit from participating in this study; however, each subject completing the study will be provided with a graphic and verbal description and explanation on their maximal aerobic capacity, heart rate, blood pressure and metabolic responses both in fasted and non-fasting states in response to different prior physical

activity/inactivity status.

Do you have to participate?

No, your participation is voluntary. You may decide not to participate at all or, if you start the study, you may withdraw at any time. Withdrawal or refusing to participate will not affect your relationship with The University of Texas at Austin (University) in anyway.

If you would like to participate please fully read, sign, and return this form to the principal investigator of this study (Heath Burton). You will receive a copy of this form for your personal records.

Will there be any compensation?

You will not receive any type of payment participating in this study.

What if you are injured because of the study?

1. The University has no program or plan to provide treatment for research related injury or payment in the event of a medical problem. In the event of a research related injury, please contact the principal investigator.

2. The University has no program or plan for continuing medical care and/or hospitalization for research-related injuries or for financial compensation.

3. If injuries occur as a result of study activity, eligible University students may be treated at the usual level of care with the usual cost for services at the Student Health Center, but the University has no program or plans to provide payment in the event of a medical problem.

How will your privacy and confidentiality be protected if you participate in this research study?

Each subject will be assigned a unique Subject ID code. This informed consent form and the Health History Questionnaire are the only places where any personal identifying information will be recorded. These forms will be stored in a locked file cabinet. In all other cases, your data will only be identifiable by your unique code. Only the director of the laboratory (Dr. Coyle) will have access to a master list that will link your identity to your code.

Because you will be participating in this study and may do so along with other subjects in a small group, we will ask that you do not disclose names of participants in your group or any information that was discussed with other group members outside of the experimental session.

If it becomes necessary for the Institutional Review Board to review the study records, information that can be linked to you will be protected to the extent permitted by law. Your research records will not be released without your consent unless required by law or a court order. The data resulting from your participation may be made available to other researchers in the future for research purposes not detailed within this consent form. In these cases, the data will contain no identifying information that could associate it with you, or with your participation in any study.

If you choose to participate in this study, you may be photographed or video recorded. Any photographs or video recordings will be stored securely and only the research team will have access to the recordings. Recordings will be kept for 3 years after the research experiment has been completed and then erased.

Whom to contact with questions about the study?
Prior, during or after your participation you can contact the researcher Heath Burton at (864)-940-4103 or send an email to heath.burton@utexas.edu for any questions or if you feel that you have been harmed.

This study has been reviewed and approved by The University Institutional Review Board and the study number is 2016-12-0022**.**

Whom to contact with questions concerning your rights as a research participant?

For questions about your rights or any dissatisfaction with any part of this study, you can contact, anonymously if you wish, the Institutional Review Board by phone at (512) 471-8871 or email at orsc@uts.cc.utexas.edu.

Participation

If you agree to participate please sign and return this form to a member of the research team.

Signature

You have been informed about this study's purpose, procedures, possible benefits and risks, and you have received a copy of this form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time. You voluntarily agree to participate in this study. By signing this form, you are not waiving any of your legal rights.

Photography and video recording of your sessions is optional. However, if participants agree to be photographed or video recorded their images may also be

used for professional and educational presentations not related to this research study.

_____ I agree to be photographed and video recorded.

_____ I do not want to be photographed and video recorded.

_____

Printed Name

_____ _____

Signature Date

As a representative of this study, I have explained the purpose, procedures, benefits, and the risks involved in this research study.

_____

Print Name of Person obtaining consent

_____ _____

Signature of Person obtaining consent Date

Appendix B: Health History Questionnaire

Health History Questionnaire

IRB #: 2016-12-0022 Subject ID: _____

Please answer the following questions to the best of your knowledge. If you do not know the answer to a specific question, please place a question mark in one of the spaces. If a question asks for more information beyond "Yes" or "No," please provide all knowledge to the best of your ability.

	Yes	No
1. Have you ever been diagnosed by a doctor as having a heart condition?	___	___
a. If yes, have you ever been advised to only perform medically supervised activity?	___	___
2. Do you have or have you ever had chest pain brought on by physical activity?	___	___
3. Have you ever been prescribed medication for a heart condition?	___	___
a. If yes, what medication? _____		
4. Are there any conditions that you are currently aware of that would prevent you from engaging in physical activity?	___	___
5. Do you smoke or have you smoked within the last (6) months?	___	___
6. Have you had any family members with a history of heart conditions?	___	___
7. Have you ever experienced shortness of breath during or after physical activity?	___	___
8. Have you ever experienced heart palpitations or an abnormally high heart rate brought on by physical activity?	___	___
9. Has a doctor ever diagnosed you with a heart murmur?	___	___
10. Do you ever experience abnormal pain in your legs during physical activity that is relieved by rest?	___	___
11. Do you have any joint or bone issues that may be aggravated by any type of exercise testing?	___	___
12. Are you currently experiencing any joint or muscle problems?	___	___
a. If yes, please describe: _____		
13. Do you or have you ever had asthma that could be aggravated by physical activity?	___	___
14. When was your last menstrual cycle? _____		
15. Do you have or have you ever had:		

a. Chronic, severe headaches ___ ___
b. Head injury (i.e. concussion, etc.) ___ ___
c. Epilepsy ___ ___
d. Abdominal pain, hernia, GI bleeding ___ ___
e. Kidney problems ___ ___
f. Anemia ___ ___
g. Hemophilia ___ ___
h. Lung problems ___ ___

16. Have you donated blood or lost a significant amount of blood within the past 2 weeks? ___ ___
17. Are you currently or have you been pregnant within the last month? ___ ___
18. Have you recently been ill? ___ ___
    a. If yes, please describe:

    _____

19. Have you been injured within the past month? ___ ___
    a. If yes, please describe:

    _____

20. Have you been injured within the past year (beyond #17) that still presents symptoms or issues? ___ ___
21. Are you currently taking any prescribed, non-cardiac related medications? ___ ___
    a. If yes, what medications?

    _____

22. Are you currently taking any over the counter medications? ___ ___
    a. If yes, what medications?

    _____

23. Do you currently train for exercise? ___ ___
    a. If yes, for how many years have you trained?

    _____

    b. If yes, what type of training do you complete?

    _____

    _____

    c. If yes, what is your general volume and intensity?

    _____

    _____

Appendix C: Blood Analysis

Plasma Glucose Measurements

Plasma glucose is measured by a spectrophotometric method using commercially available kits (Pointe Scientific, Inc. Canton, USA). The plasma samples are removed from freezer (-80°C) and thawed in an ice-water bath. 3 µL of plasma sample is added to 300 µL of glucose reagent and then incubated at room temperature for 10 minutes, 5 minutes of which are on an oscillating tray. Glucose is phosphorylated with ATP to produce glucose 6-phosphate (G-6-P) in the reaction catalyzed by hexokinase (HK). The glucose 6-phosphate is then oxidized with concomitant reduction of NAD to NADH in the reaction catalyzed by glucose 6-phosphate dehydrogenase (G6PDH). The absorbance of NADH formed will be measured at 340 nm using a microplate reader (Tecan Infinite 200 PRO, Tecan Group Ltd., Männedorf, Switzerland). The concentration of NADH is directly proportional to the concentration of glucose in the sample.

Plasma Triglyceride Measurements

Plasma triglyceride will be measured using a spectrophotometric method using a commercially available kit (Pointe Scientific, Inc., Canton, USA). When beginning analysis, plasma samples are removed from the freezer and thawed at room temperature and vortexed. 3 µL of plasma is then pipetted off and added to 300 µL of pre-warmed (37°C) triglyceride reagent. These samples are and incubated for 5-minutes on an oscillating tray. The plate is then moved to a warm (37°C) to finish incubating for 20-minutes. The reagent used hydrolyzes triglycerides in the sample via lipase and produces glycerol and free fatty acids. Glycerol is then phosphorylated by ATP to glycerol 1-phosphate and ADP through a reaction catalyzed by glycerol kinase (GK). The glycerol 1-phosphate is then oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen

peroxide. The condensation of hydrogen peroxide with 4-chlorophenol and 4-aminophenazone (4-AA) in the presence of peroxidase (POD) produces a red colored quinonimine dye. The intensity of the colored complex formed is directly proportional to the triglycerides concentration of the sample. The plate is read at 500 nm using a microplate reader (Tecan Infinite 200 PRO, Tecan Group Ltd., Männedorf, Switzerland).

Appendix D: Raw Data

D.1 Biographical, VO_{2MAX}, and Resting Metabolic Rate Data

Subject #	Biographical Data			VO_{2max}		
	Age (y)	*Height (m)*	*Weight (kg)*	*VO_{2max} (Absolute)*	*VO_{2max} (Relative)*	*RMR (kcal/day)*
1	25	1.71	77.70	2562.55	32.98	1438.18
2	21	1.57	55.80	1872.98	33.57	1358.16
3	25	1.70	70.70	4369.26	61.80	2553.68
4	34	1.76	114.55	3680.84	32.13	2591.71
6	21	1.64	59.71	2710.63	45.40	2156.09
7	22	1.85	123.03	5350.57	43.49	3549.00
8	24	1.64	67.50	3421.42	50.69	1757.83
9	23	1.87	77.83	4295.94	55.20	2842.13
10	23	2.01	127.95	5318.37	41.57	2825.00
11	20	1.56	55.35	2075.63	37.50	1949.64

D.2 Daily Steps and Postural/Activity Data

Subject #	Day of Trial						
	C1	C2	D1	D2	D3	D4	D5
SIT							
1	12674	13120	6638	3818	2416	2768	874
2	8788	7610	6516	2454	1744	2782	1984
3	4074	5726	4102	2596	2318	3270	970
4	10810	2902	6212	4812	6034	3580	1336
6	6180	6180	1798	4252	4858	2846	588
7	4180	2170	5844	5562	4902	7450	320
8	2262	4278	2896	5496	1978	3450	52
9	7538	5336	2130	2266	4890	2408	124
10	7586	9024	3854	3028	3082	1624	1092
11	5130	3628	2682	996	6254	4490	2210
SIT+EX							
1	14714	12914	7136	5160	2632	10924	618
2	7603	7164	3768	3252	3156	13756	836
3	5486	5842	2836	2608	3062	13900	1056
4	8746	7980	5840	6882	6888	10480	798
6	6698	6498	3544	5264	4270	13472	1472
7	5196	6142	4590	5532	5900	15784	320
8	7904	3973	3580	2243	1981	1305	775
9	7158	6080	1456	1762	3138	10636	846
10	4936	5430	4900	2722	3388	10928	78
11	5948	3684	3084	4892	4094	11536	496

Subject #	Day of SIT Trial						
	C1	C2	D1	D2	D3	D4	D5
Sitting (min/day)							
1	1062	936	913.2	1274.4	1266	1302.6	832.2
2	1236	1300.8	1060.8	1356	1359	1302.6	776.4
3	1247.4	1291.2	1354.8	1377	1333.2	1322.4	848.4
4	1115.4	1318.2	1182	1278	1156.8	1255.8	490.8
6	1266.6	1300.2	1366.2	1213.2	1161	1305	375
7	1321.8	1363.8	1248.6	1248	1259.4	1416.6	490.2
8							
9	1273.2	1321.2	1365.6	1335	1330.2	1336.2	465.6
10	1176.6	1224.6	1243.2	1256.4	1265.4	1267.8	464.4
11	1165.2	1182.6	1344	1302	1232.4	1131.6	832.8
Standing (min/day)							
1	216.6	324.6	448.8	117.6	141	100.2	70.2
2	109.2	63	307.2	57	60.6	103.2	16.2
3	134.4	82.8	40.2	31.8	73.8	73.2	10.2
4	203.4	87	175.2	100.8	204	138	19.8
6	103.2	76.8	49.2	169.2	217.2	96	34.2
7	67.8	46.2	118.8	119.4	129.6	21	11.4
8							
9	70.2	55.2	46.8	76.2	52.8	72.6	9
10	167.4	115.2	145.8	140.4	132.6	146.4	59.4
11	205.8	209.4	61.8	124.2	135.6	249	48
Stepping (min/day)							
1	162	179.4	78	48	33	36.6	12.6
2	94.8	76.2	71.4	27	20.4	34.2	12.6
3	58.2	66	45	31.2	33	44.4	10.8
4	121.8	34.8	82.8	61.2	78.6	46.2	17.4
6	70.2	63	24	57.6	61.8	39	7.2
7	50.4	30	72.6	72.6	51	2.4	0
8							
9	88.2	63.6	27.6	28.8	57	31.8	1.8
10	96.6	100.2	51.6	43.2	42	25.2	13.2
11	69	48	34.2	14.4	72	59.4	23.4

Subject #	Day of SIT+EX Trial						
	C1	C2	D1	D2	D3	D4	D5
Sitting (min/day)							
1	959.4	980.4	1282.8	1217.4	1279.2	1126.8	837
2	1414.8	1267.2	1303.8	1293.6	1150.8	1161	826.8
3	1213.8	1137	1211.4	1311	1314	1203.6	492.6
4	1063.2	1101	974.4	1226.4	1133.4	1413.6	572.4
6	1101	1288.8	1199.4	1261.2	1294.8	1162.2	707.4
7	1298.4	941.4	1290.6	1259.4	1173.6	1165.8	536.4
8							
9	1262.4	1283.4	1394.4	1390.2	1285.8	1309.8	853.8
10	1155	1240.8	1216.8	1323.6	1116	1311.6	1372.2
11	1097.4	1218.6	1297.8	1180.8	1229.4	1162.2	832.2
Standing (min/day)							
1	304.8	308.4	75	159.6	126	204	46.8
2	16.8	95.4	94.8	107.4	253.8	157.2	22.8
3	157.8	225.6	190.2	91.8	88.8	118.2	16.2
4	261.6	235.8	391.8	128.4	220.8	19.8	14.4
6	258.6	75.6	192	116.4	90	154.8	27
7	136.8	418.8	88.8	106.8	257.4	124.8	6.6
8							
9	95.4	80.4	27.6	28.2	114.6	46.8	15
10	214.2	127.2	163.8	81	270.6	40.2	66
11	267	171.6	105	200.4	156.6	169.2	25.8
Stepping (min/day)							
1	175.8	151.8	81.6	63	35.4	109.2	9.6
2	8.4	77.4	41.4	39	35.4	121.8	9.6
3	68.4	77.4	38.4	37.8	37.2	118.8	12
4	115.2	102.6	73.8	85.2	85.8	6.6	9.6
6	80.4	76.2	48.6	62.4	55.2	122.4	16.8
7	4.8	80.4	60.6	73.8	9	149.4	4.2
8							
9	82.2	76.2	18	21.6	39.6	83.4	9.6
10	70.8	72	59.4	35.4	53.4	88.2	1.2
11	75.6	49.2	37.2	58.8	54	108.6	7.2

D.3 CALORIC INTAKE

Subject #	Day of Trial						
	C1	C2	D1	D2	D3	D4	D5
SIT							
1	1441	1246	1300	1350	1181	1519	1271
2	1089	965	1155	622	912	1416	962
3	2105	2105	2105	2105	2370	2560	1275
4	2140	2055	2372	1747	1875	2595	1889
6	1819	1563	1933	1510	1935	1955	1004
7	3580	3670	2740	3740	3630	2845	1953
8	1778	1773	1786	1798	1792	1760	1255
9	3003	3045	1322	1855	2444	2705	1258
10	2745	2757	2215	2816	3059	2805	2053
11	1090	1443	880	1190	1455	1845	994
SIT+EX							
1	1441	1246	1270	1350	1088	1939	1275
2	969	965	1319	815	689	1836	964
3	2105	2370	2060	2145	2060	3370	1256
4	2140	2055	2372	1747	1875	3135	1886
6	1819	1563	1933	1510	1935	2495	1004
7	3580	3670	2740	3740	3630	3885	2005
8	1772	1772	1754	1714	1721	2410	1269
9	3003	2995	1322	1855	2444	3515	1253
10	2764	2910	2265	2488	3164	3885	1975
11	1090	1443	880	1190	1455	2183	1000

D.4 POSTPRANDIAL RESPONSES (PLASMA)

Subject #	Plasma Triglyceride Concentration (mg/dL)						
	Baseline	H1	H2	H3	H4	H5	H6
SIT							
1	41.01	71.44	99.54	84.15	95.94	90.51	69.90
2	79.61	99.45	76.64	101.35	101.16	95.60	89.46
3	56.51	130.60	192.90	169.74	214.54	225.36	179.25
4	205.53	228.89	338.29	418.86	395.85	353.56	359.10
6	56.16	83.82	115.82	152.99	145.45	100.35	89.23
7	81.59	170.93	227.58	228.79	195.72	149.42	228.74
8	60.85	102.53	105.03	95.81	96.57	93.84	97.33
9	69.83	76.74	133.88	192.60	155.99	145.11	119.99
10	50.33	112.95	173.58	210.90	191.96	152.75	151.01
11	47.28	109.79	143.23	87.61	86.68	87.85	87.22
SIT+EX							
1	39.66	75.29	95.88	115.43	130.64	121.67	104.80
2	127.16	82.55	100.99	58.53	67.38	90.84	95.00
3	85.79	146.14	202.35	186.10	139.12	147.09	57.44
4	151.32	185.93	217.35	279.23	304.21	228.89	252.48
6	46.55	60.76	87.20	112.68	89.42	95.03	31.51
7	120.94	226.41	308.66	283.57	298.98	331.78	423.10
8	53.79	83.47	95.51	93.80	84.20	66.13	73.83
9	70.32	115.40	104.48	138.57	168.93	117.47	104.27
10	55.50	100.22	170.54	193.06	187.54	142.76	114.39
11	133.08	90.36	91.02	86.97	100.03	80.98	70.79

Subject #	Plasma Glucose Concentration (mg/dL)						
	Baseline	H1	H2	H3	H4	H5	H6
SIT							
1	79.03	96.01	96.61	103.81	114.37	116.64	87.06
2	106.25	123.85	85.25	127.94	119.62	149.39	149.93
3	116.50	102.31	110.57	98.62	113.46	139.26	123.96
4	94.38	124.82	128.26	197.07	187.26	146.71	113.67
6	85.49	76.40	100.74	83.28	71.41	66.40	79.47
7	69.61	93.03	110.94	107.85	114.93	84.69	85.49
8	105.81	82.22	97.40	113.37	137.23	146.31	106.21
9	120.05	85.09	79.70	117.45	133.95	112.49	104.21
10	103.74	111.66	131.61	116.36	99.46	129.55	102.66
11	85.39	93.14	114.94	136.51	137.47	140.88	89.37
SIT+EX							
1	58.67	59.81	99.67	109.30	104.28	96.77	81.04
2	149.14	105.00	137.66	144.28	124.14	126.28	115.90
3	120.16	126.97	164.86	156.72	137.05	169.38	88.17
4	104.16	136.24	126.70	141.96	163.73	131.43	102.96
6	80.84	53.59	65.17	107.62	99.36	81.05	55.10
7	93.74	90.82	146.14	94.23	124.47	123.95	133.33
8	96.63	83.74	104.12	150.66	172.80	135.84	107.76
9	129.83	107.29	82.37	85.37	87.26	104.62	102.23
10	93.39	88.31	107.95	98.59	130.32	168.48	108.82
11	97.20	64.79	113.94	122.01	146.24	121.49	84.20

D.5 POSTPRANDIAL RESPONSES (GAS)

Subject #	Respiratory Exchange Ratio			
	Baseline	H2	H4	H6
SIT				
1		1.01	1.08	1.01
2		0.87	0.97	0.79
3	0.80	0.81	0.78	0.73
4	0.73	0.84	0.76	0.72
6	1.07	1.00	1.01	0.77
7	1.20	1.03	1.09	1.01
8	0.78	0.89	0.78	0.74
9	1.00	1.02	1.30	1.29
10	0.80	0.82	0.74	0.72
11	0.80	0.81	0.78	0.77
SIT+EX				
1		0.99	1.01	1.04
2		0.84	0.82	0.80
3	0.73	0.80	0.73	0.76
4	0.85	0.77	0.74	0.78
6	0.83	0.85	0.80	0.80
7	1.24	1.06	1.00	1.03
8	0.83	0.86	0.75	0.73
9	1.22	1.21	1.17	1.13
10	0.73	0.78	0.71	0.70
11	1.25	1.19	1.12	1.03

Subject #	%Carbohydrate Oxidation			
	Baseline	H2	H4	H6
SIT				
1		105.05	127.17	104.44
2		55.63	91.13	26.96
3	30.03	36.52	24.23	6.48
4	9.22	46.42	16.38	3.41
6	123.21	98.63	104.44	20.82
7	168.60	109.90	131.40	103.99
8	24.57	62.12	24.91	10.92
9	99.62	106.14	200.68	197.95
10	30.03	37.20	10.58	3.96
11	30.03	35.49	24.37	22.18
SIT+EX				
1		96.25	102.73	114.61
2		44.71	39.25	32.08
3	7.17	32.08	6.83	18.77
4	48.12	19.80	9.56	23.89
6	40.96	49.49	32.08	33.11
7	181.57	120.48	101.02	109.90
8	40.27	50.85	14.57	8.36
9	173.38	172.35	159.04	144.37
10	6.83	25.94	0.99	-3.48
11	185.32	165.19	141.98	109.83

Subject #	%Fat Oxidation			
	Baseline	H2	H4	H6
SIT				
1		-5.05	-27.17	-4.44
2		44.37	8.87	73.04
3	69.97	63.48	75.77	93.52
4	90.78	53.58	83.62	96.59
6	-23.21	1.37	-4.44	79.18
7	-68.60	-9.90	-31.40	-3.99
8	75.43	37.88	75.09	89.08
9	0.38	-6.14	-100.68	-97.95
10	69.97	62.80	89.42	96.04
11	69.97	64.51	75.63	77.82
SIT+EX				
1		3.75	-2.73	-14.61
2		55.29	60.75	67.92
3	92.83	67.92	93.17	81.23
4	51.88	80.20	90.44	76.11
6	59.04	50.51	67.92	66.89
7	-81.57	-20.48	-1.02	-9.90
8	59.73	49.15	85.43	91.64
9	-73.38	-72.35	-59.04	-44.37
10	93.17	74.06	99.01	103.48
11	-85.32	-65.19	-41.98	-9.83

Subject #	Energy Expenditure (kcal/min)			
	Baseline	H2	H4	H6
SIT				
1		1.24	1.23	1.36
2		1.23	1.33	1.17
3	1.35	1.74	1.54	1.54
4	1.68	2.12	1.95	1.49
6	1.33	1.61	1.39	1.34
7	2.32	2.39	1.82	2.70
8	1.24	1.48	1.45	1.40
9	1.61	2.07	2.24	2.17
10	1.89	2.41	2.24	2.26
11	1.12	1.17	1.19	1.22
SIT+EX				
1		1.52	1.46	1.43
2		1.16	1.12	1.05
3	1.47	1.86	1.56	1.52
4	1.30	2.00	1.93	1.91
6	1.42	1.73	1.47	1.42
7	2.49	2.67	2.63	2.61
8	1.32	1.52	1.51	1.37
9	1.83	2.11	2.14	2.06
10	2.00	2.41	2.55	2.21
11	1.36	1.56	1.47	1.34

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